A FACTORIAL ANALYSIS OF DISTRIBUTION IN THREE SPECIES OF DIPLOPODS

BY

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INTRODUCTION

All animal species tend to spread by nature of their innate activities. If this dispersal depended only on activity potentials, all species would increase in range, each at its own characteristic rate, until they extended over the whole earth. In reality, the distributions of different species vary tremendously because of the influence of certain checks or limiting factors consisting of the complex of biotic and abiotic elements in their environments. A comparative study of the distributions of different species necessitates examination of the reactions of animals to these environmental elements which determine their distributions. In an analysis of these reactions, it is usual, because of the complexity of interacting factors, to study the effect of each factor individually. With the information thus obtained, a synthesis of causal relationships is attempted in an effort to achieve understanding of the coördinated influences of all agents affecting the animals simultaneously under natural conditions. However, in ecology the whole may be greater or less than the sum of its parts. The effect of one factor upon an animal may be augmented or suppressed by the concomitant action of another. The level of intensity at which each factor alone becomes limiting may be altered by the combined influence of all the factors. Consequently, a study of the effects of single agents must be regarded with a certain amount of reserve and as possibly only a first step towards understanding the coördinated effects of all the influences combined.

The most favourable circumstances for analysis of the relations between animals and their surroundings are those in which different combinations of biotic and abiotic factors are simplest and most clearly distinguishable. In this respect, sand dunes offer admirable situations for ecological studies. The structural character of this type of terrain, its knolls, plateaus, valleys, and pools and its vegetation, produce the greatest number of observably different habitats in the smallest possible area.

Ecological studies in the dune area Meijendel, near Leiden, were undertaken at the Zoology Laboratory of the University of Leiden in 1953. The purpose of this project is to determine where different species are found and what the conditions are, in these places, which may account for apparent preferences of certain species for different habitats and, thirdly, to determine, by laboratory experiments, what the reactions of the animals towards various factors are and whether differences between these reactions in different species are sufficient to explain observed diversity in their distributions. The dune area Meijendel is particularly acceptable for this work because, since 1874, it has been used as a water reservoir for The Hague and, as a consequence of this function, the history of the land and variations in the underground water level are well known.

The present study, which represents part of the Meijendel project, is an evaluation of humidity, temperature, and food, as limiting factors in the distributions, in the dunes, of three species of millipedes: *Iulus scandinavius* Latz., *Cylindroiulus silvarum* (Mein.) and *Schizophyllum sabulosum* (L.).

GENERAL DISTRIBUTION AND BIOLOGY OF THE SPECIES

Although the distributions of these three species in Europe are fairly well known, little detailed information on their ecologies is available. SCHUBART (1934) has given a summary, which I have combined with information of my own to produce the following survey.

Iulus scandinavius Latz.

DISTRIBUTION. North and central France and Alsace, Switzerland, Belgium and Holland, the British Isles, Denmark, southern Norway and southern Sweden, western Hungary, Austria, Czechoslovakia.

BIOLOGY. A species typical in humid, densely wooded areas; also occasional under stones; it avoids cultivated ground. My observations indicate that mating takes place chiefly in late spring but may continue through the summer. Oviposition occurs certainly in late autumn or early winter, in Holland, and perhaps also in late summer.

Cylindroiulus silvarum (Mein.).

DISTRIBUTION. France, Holland and Belgium, the British Isles, Denmark, Norway, along the coast of Sweden, Finland, northwest Poland, and introduced into Newfoundland (LINDROTH, 1957, p. 110).

BIOLOGY. Typical in wooded terrain, deciduous as well as coniferous, especially on ground covered by a thick layer of fallen leaves. Commonly found in and under rotting wood. Oviposition probably occurs in late summer or autumn but mating may take place during the whole year. Animals brought into the laboratory during the winter copulate readily.

Schizophyllum sabulosum (L.).

DISTRIBUTION. Spain, France, Switzerland, Belgium and Holland, the British Isles, Denmark, southern Norway, Sweden, Finland, Russia: as far as the southern Urals, Estonia, Latvia, Lithuania, Poland, Rumania, Yugoslavia, Albania, Hungary, Austria, Czechoslovakia, Italy: as far south as Rome, San Marino.

BIOLOGY. A eurytopic species occurring in dry, hilly areas of open sand and poor vegetation as well as in both deciduous and coniferous woods; it avoids cultivated terrain. In Holland, the species is active from May to October during which time it can often be found climbing small trees and bushes at night. SCHUBART (l.c.) has noted that the males disappear in autumn before the females but mentioned nothing about reproduction in this species.

DISTRIBUTION

The word "distribution", as applied to animals, embraces two different aspects of dispersal. In its broadest sense, the term connotes the total area over which a species exists. This area, although not absolutely constant, undergoes modification relatively slowly because it is dependent largely on geographical and meteorological conditions which are continental in proportion and which change very gradually. This conception of range may be referred to as "geographical distribution".

Secondly, even within the normal range of a species, it never occurs in every locality within the range because, although a species tends to spread as widely as possible, its dispersion is checked by various limiting factors which prevent it from living successfully everywhere and confine it to certain definite situations. This restriction of a species to more or less defined habitats within its total range has been termed "ecological distribution" by UVAROV (1931). Subsequent use of the word distribution will imply this idea of range unless otherwise stated.

Distribution may be regarded in terms of time as well as space in the sense that the activity of individuals of a species, and thus their occurrence in different localities, may vary during the year. The approach of winter affects practically all insects (UVAROV, l.c.) and innumerable other animals, changes in environmental conditions, in a temperate or arctic climate, producing some form of quiescent state in the animals. Besides the changes in activity concomitant with overwintering, fluctuations obviously connected with the season may take place in the behaviour of fully active insects (ROUBAUD, 1909; SWYNNERTON, 1921: MYERS, 1926; JACK, 1927).

In the following analysis, both the distributions of the three species of millipedes in a definite area and their seasonal activities will be considered.

METHOD

To determine the distributions of these millipedes, as well as other arthropods considered in the Meijendel project, a method of trapping is used employing, as traps, metal boxes with dimensions of 24 × 24 × 27 cm., which are buried in the ground up to the top rims. A screen-covered hole in the bottom of each box provides for drainage of rainwater. To reduce the amount of water, leaves, sand, etc., which tend to collect in the traps, metal roofs of 30 × 30 cm. are placed over them. These covers stand on metal legs holding them several cm. above the surface of the ground. Although certain animals might conceivably be attracted to such a shelter, experiments to investigate this possibility indicated that the roof does not influence the catch.

One hundred of these traps are placed in groups of three or six in various, obviously different, types of terrain. These habitats include, on the one hand, bare sand quite near the sea, to relatively old, fairly dense, wooded terrain on the other hand, with various habitats intermediate in succession between these two extremes. Descriptions of these different terrains, on a macrohabitat level, are given below and have previously been presented by DEN BOER (1956).

SERIES 1. — In the so-called "sea dunes". Very rough, relatively bare dunes with one or two groups of small trees.

(a) six traps in largely bare sand (1—6),
(b) six traps in a depression carpeted by mosses, lichens, and short grass with here and there a few creeping willows (*Salix repens* L.) (7—12),
(c) three traps in each of two small woods consisting of short birches (*Betula verrucosa* Ehrh.) and situated relatively near the sea (13—15, 22—24),
(d) three traps outside each of the woods in (c); three in bare sand with patches of the grass, *Ammophila arenaria* (L.) Link., (16—18) and three in a thick growth of *Calamagrostis epigejos* (L.) Roth., (19—21).

SERIES 2. — Similar to series 1 but farther from the sea. Largely bare sand with a few groups of small trees or bushes.

(a) on a plain thickly covered with mosses, lichens, and short grass (61—63),
(b) in bare sand (64—66),
(c) in a growth of *C. epigejos* under scattered birches (67—69),
(d) under creeping willows (70—72),
(e) on a hillside among a number of old, black poplars (*Populus nigra* L.) (73—75).

SERIES 3. — Woods situated in a steep-walled depression among rough terrain. About equal distance from the sea as series 2.

(a) on a small plain overgrown by *C. epigejos* (76—78),
(b) two groups of three traps each in dense birch woods (79—81 and 82—84),
(c) four traps in an open terrain outside the wooded area in (b); sand overgrown by mosses, lichens, and short grass with a few creeping willows (85—87, 100).
(d) six traps on an open plateau formed by excavated sand and covered by *A. arenaria* and *C. epigejos*; about four m higher than the woods in (b) (88—93),
(e) in a moist birch wood with a rich and varied undergrowth of such plants as *Mentha aquatica* L., *Valeriana officinalis* L., and *Eupatorium cannabinum* L., (94—96),
(f) in isolated, dense, birch woods (97—99).

SERIES 4. — A relatively old, wooded terrain in a completely flat area of dunes lying about two km from the sea.

(a) on a small plain, three traps in a stretch of largely bare sand (28—30), and three in an area partly overgrown by *A. arenaria* (25—27),
(b) in dense aspen woods (*Populus tremula* L.) (31—33),
(c) in open terrain covered by a dense growth of *C. epigejos* (34—36),
(d) largely open terrain overgrown by *C. epigejos* and *Hypericum perforatum* L., with scattered birches and *Crataegus monogyna* Jacq. (37—39),
(e) among a patch of thorn bushes, *Hippophaë rhamnoides* L. (40—42),
(f) in a stand of aspen trees with a thick undergrowth of nettles, *Urtica dioica* L. (43—45),
(g) a flat area densely overgrown by *C. epigejos* with scattered birches merging into the woods in (f) (46—48),
(h) a clearing in (f) covered by *C. epigejos* (49—51),
(i) in an extension of (f) consisting of aspen trees with a thick undergrowth of *Humulus lupulus* L., and honeysuckle, *Lonicera periclymenum* L. (52—54),

(j) a wooded area in the same complex and similar to (i) but with a more varied undergrowth; three traps in a depression (55—57) and three on ground about two m higher (58—60).

Once a week the animals trapped in each box are removed and preserved in alcohol for later identification, counting, and sexing. Examination of results obtained by this method of sampling embodies several different considerations. Primarily, the number of individuals of a species which is caught in a certain trap is dependent on the total number of animals present in the area around the trap. Thus, comparing the number of animals of each species which are caught in different habitats indicates their relative distribution in the experimental area. But the catch is also dependent on the activity of the animals. This activity depends on characteristics of the species and is modified by factors external to the animal such as: temperature, humidity, sunshine, rain, etc., and by internal factors such as hibernation and reproductive periodicity. Results thus evidence not only habitat preference but also the influence of environmental factors on activity as well as cyclic changes in activity during the year.

Analysis of trappings in relation to weather conditions has been facilitated by the use of daily weather reports issued by a station of the government weather bureau fortunately situated only a few km from the test area.

RESULTS AND DISCUSSION

Distribution

The total number of individuals of each species which was trapped per year in each habitat during three successive years is shown in Figs. 1, 2, and 3. Three different types of distribution are illustrated. *C. silvarum* has a relatively narrow range in the area, being limited entirely to a comparatively old terrain of birch and aspen woods with here and there stretches of shrubbery and a few grassy clearings. *I. scandinavius* has a much wider distribution and has been trapped in most of the habitats sampled in the experimental area. Although, like *C. silvarum*, it typically occurs in greatest numbers in woods (traps 13—15, 33—60, 79—84, 94—99), it extends its range, unlike *C. silvarum,* to open, grassy places (19—21, 76—78, 88—93) and sometimes occurs even in stretches of nearly bare sand (1—9, 85—87). A third type of distribution is shown by *S. sabulosum*. This is a species characteristic of open terrain rather than woods and its distribution pattern shows relatively little discrimination between different habitats.

Comparing these results with the geographical distributions of the three species, which were summarized in the introduction, gives the same general impression as regards *S. sabulosum*. This species has a much wider total range than *I. scandinavius* and *C. silvarum*; it has been reported in 24 countries and extends into mountains as well as into semi-tropics. SCHUBART (1934) also described *S. sabulosum* as a species equally common in semi-deserts and in forests. Conversely, *I. scandinavius* and *C. silvarum* may have very different ecological distributions, as in this case, but, perhaps because they are both typical forest species, their geographical distributions are similar, *I. scandinavius* being slightly more widespread (reported in 13 countries) than *C. silvarum* (11 countries). These facts illustrate the essential difference which may be implied between the terms ecological and geographical distribution.

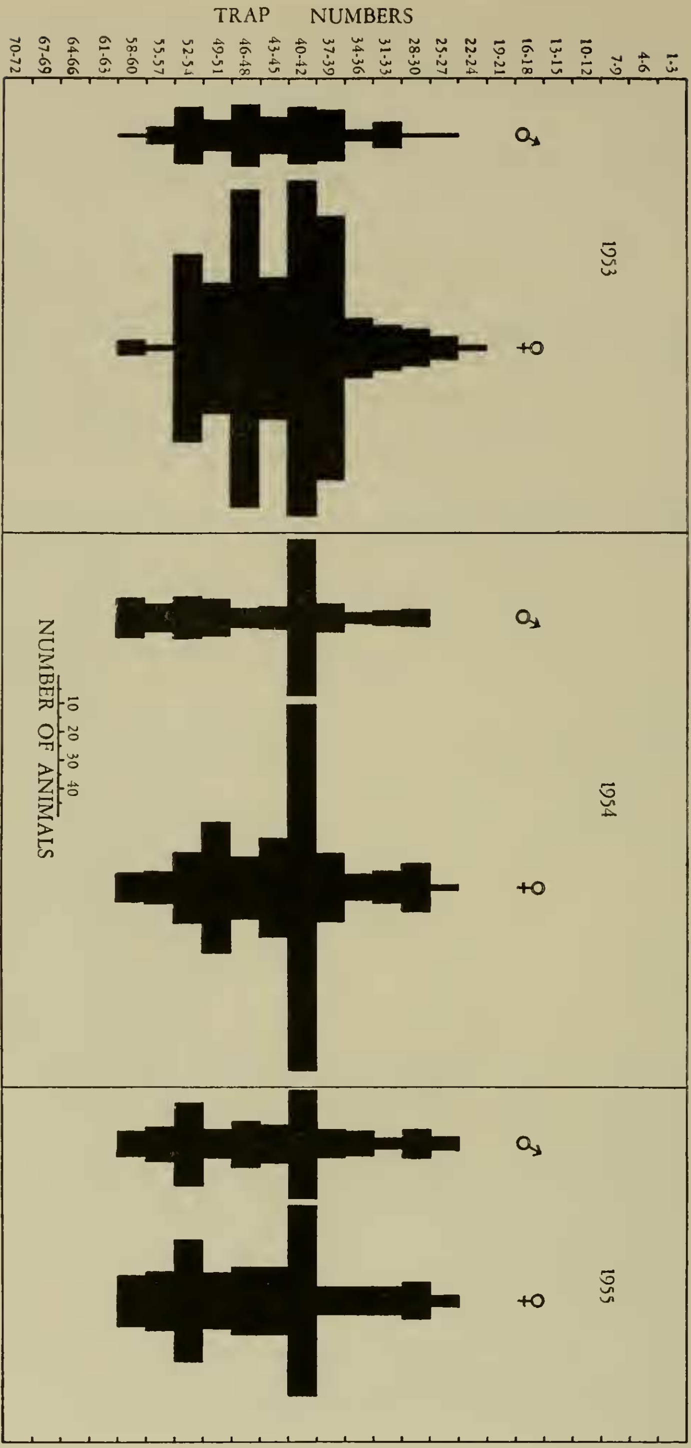


Fig. 1. Distribution of *C. silvarum.* Total number of animals per year trapped in different habitats. For description of habitats, see text.

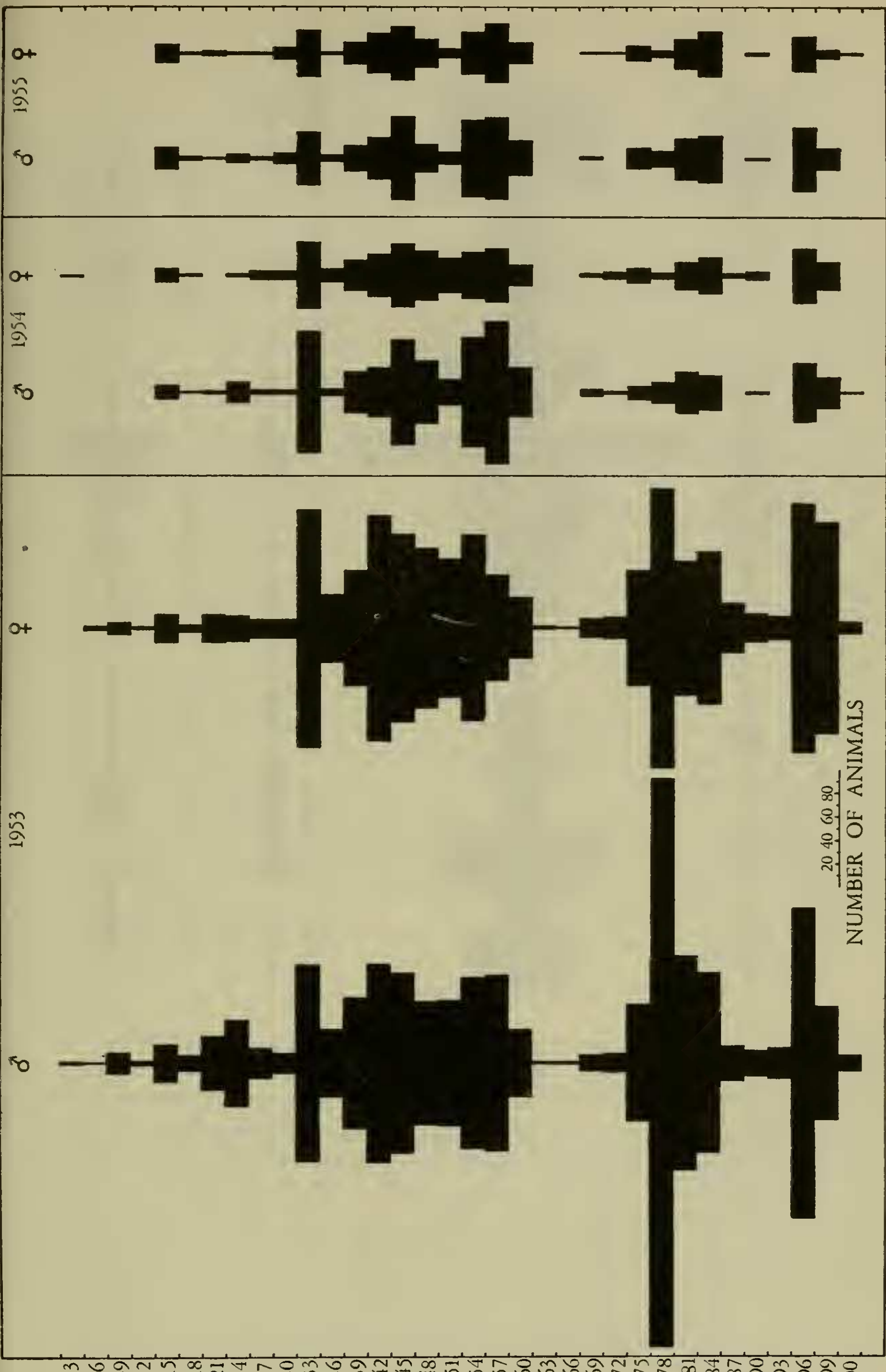


Fig. 2. Distribution of *I. scandinavius*. Total number of animals per year trapped in different habitats. For description of habitats, see text.

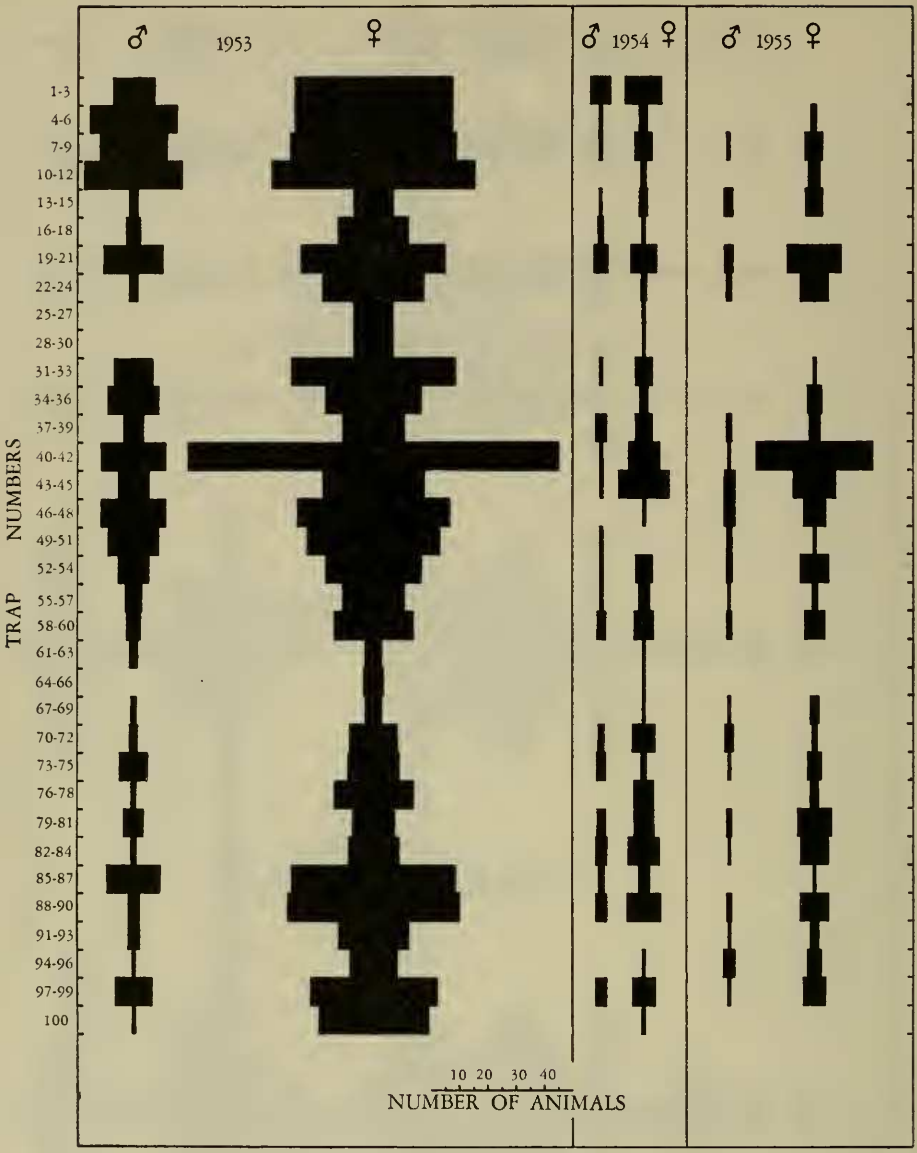


Fig. 3. Distribution of *S. sabulosum*. Total number of animals per year trapped in different habitats. For description of habitats, see text.

Figs. 1—3 show further that the distribution patterns, particularly of *I. scandinavius* and *C. silvarum,* were fairly uniform over the three years which have been analysed. Although the total number of individuals of any one species varied in different years, the relative abundance of the species in different habitats was comparable from year to year. The distribution of *S. sabulosum* in the area was somewhat less constant over the three years. This inconsistency further indicates the absence of a pronounced preference for any particular habitats on the part of this species.

The most aberrant distribution patterns were obtained in 1953, a year in which the population of each species in the area apparently reached massive proportions. No attempt will be made here to explain this phenomenon of periodic outbreaks of populations which seems to be typical of millipedes and which is probably much more frequent in many species than is generally realized. The causes of such outbreaks, which have been discussed by Uvarov (1931), may be numerous and complex and may depend on the suitability of conditions not only during the time of outbreak but also during previous years. The distributions, particularly of *I. scandinavius* and *S. sabulosum,* during 1953 appeared to be somewhat wider than in the two subsequent years. The explanation for this may be twofold. It is feasible that the abnormally abundant populations increased the chances that individuals would be trapped in any particular habitat. Secondly, it is possible that the ranges were, in fact, wider during the year of outbreak. Increased freedom ecologically is evidently a feature of outbreaks of many species of insects. (Uvarov, l.c.) and has been cited for locusts by Predtetchenskii (1928).

Intraspecifically, females of *C. silvarum* and *S. sabulosum* often showed slightly wider distributions than males of the same species. This fact, again, might conceivably reflect the greater abundance of females which could have increased their chances of being trapped in any given habitat. The distributions of male and female *I. scandinavius* were about the same and the numbers of individuals of each sex which were trapped were also similar. However, it is possible too that males of *C. silvarum* and *S. sabulosum* are actually slightly more restricted ecologically than the females. Evidence to substantiate this argument will be found in subsequent sections of this paper.

Seasonal Activity

The monthly activities of the millipedes can be seen in Figs. 4, 5, and 6 in which the total numbers of individuals trapped each month are recorded. All three species were relatively inactive during the winter months. In *I. scandinavius* and *S. sabulosum* overwintering is accomplished by a vertical migration into the ground. During winter individuals of these two species can be found from one or two cm under the surface of the ground, to a depth of sometimes ½ m in places where the soil is loose. *C. silvarum* can be found likewise in the ground during winter but this species overwinters also under the bark of logs lying on the surface of the ground in woods. The mechanism of overwintering will be discussed under consideration of humidity as an environmental factor.

Besides the winter inactivity common to the three species, there were also variations in activity throughout the favourable part of the year. It was in this

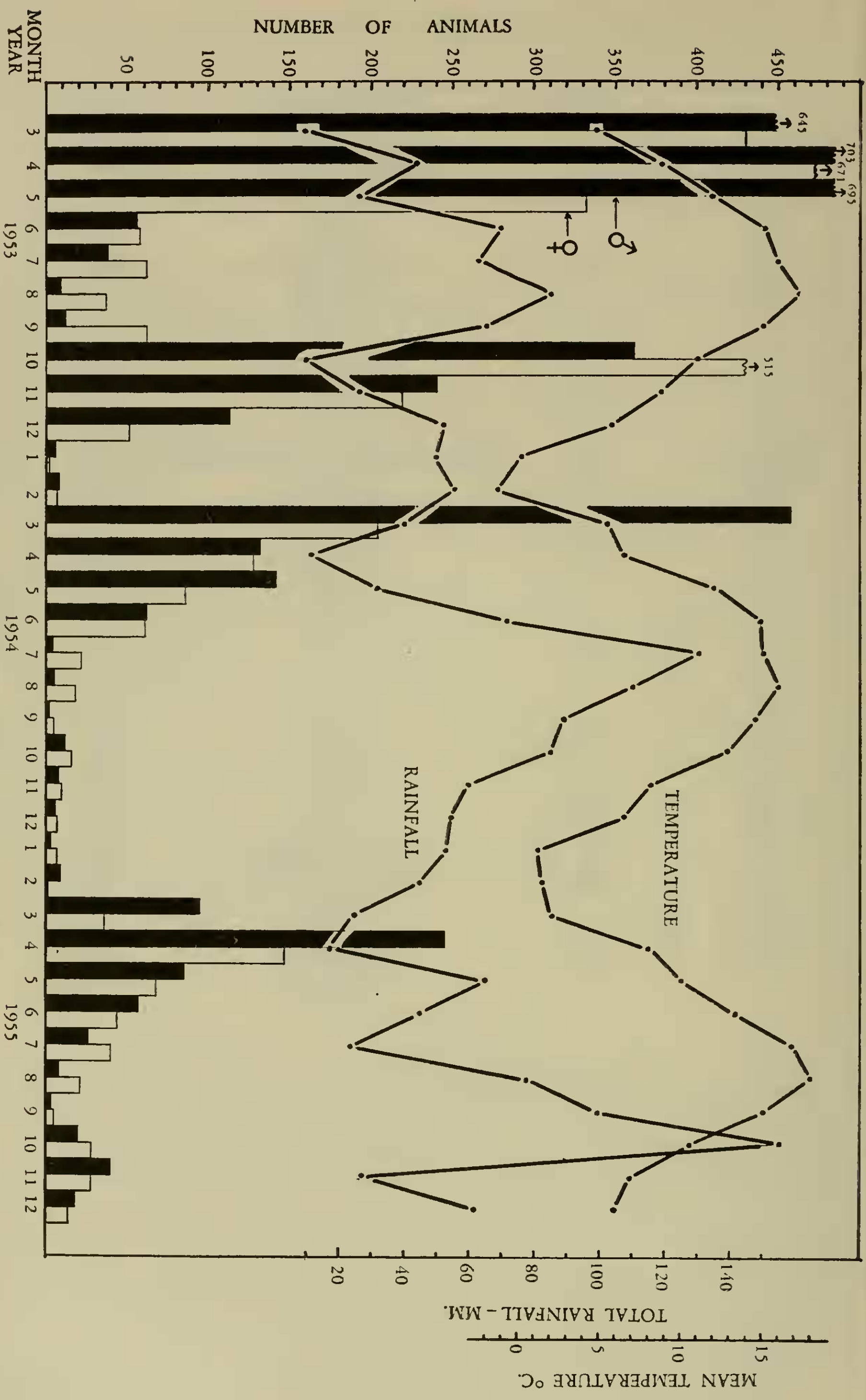


Fig. 4. Seasonal activity of *I. scandinavius* with mean monthly temperature and total precipitation per month. Columns represent total number of animals trapped in all habitats.

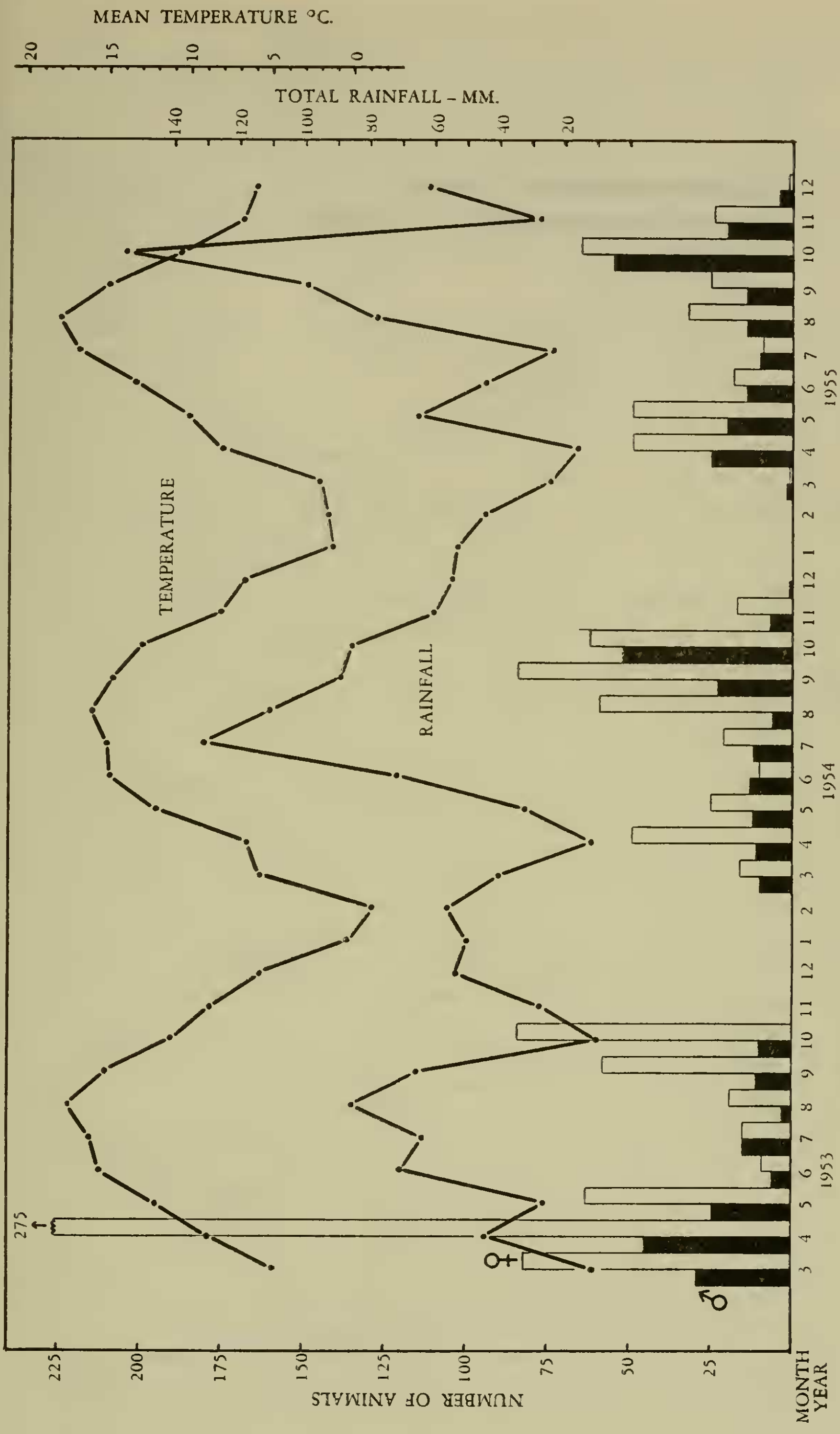


Fig. 5. Seasonal activity of *C. silvarum* with mean monthly temperature and total precipitation per month. Columns represent total number of animals trapped in all habitats.

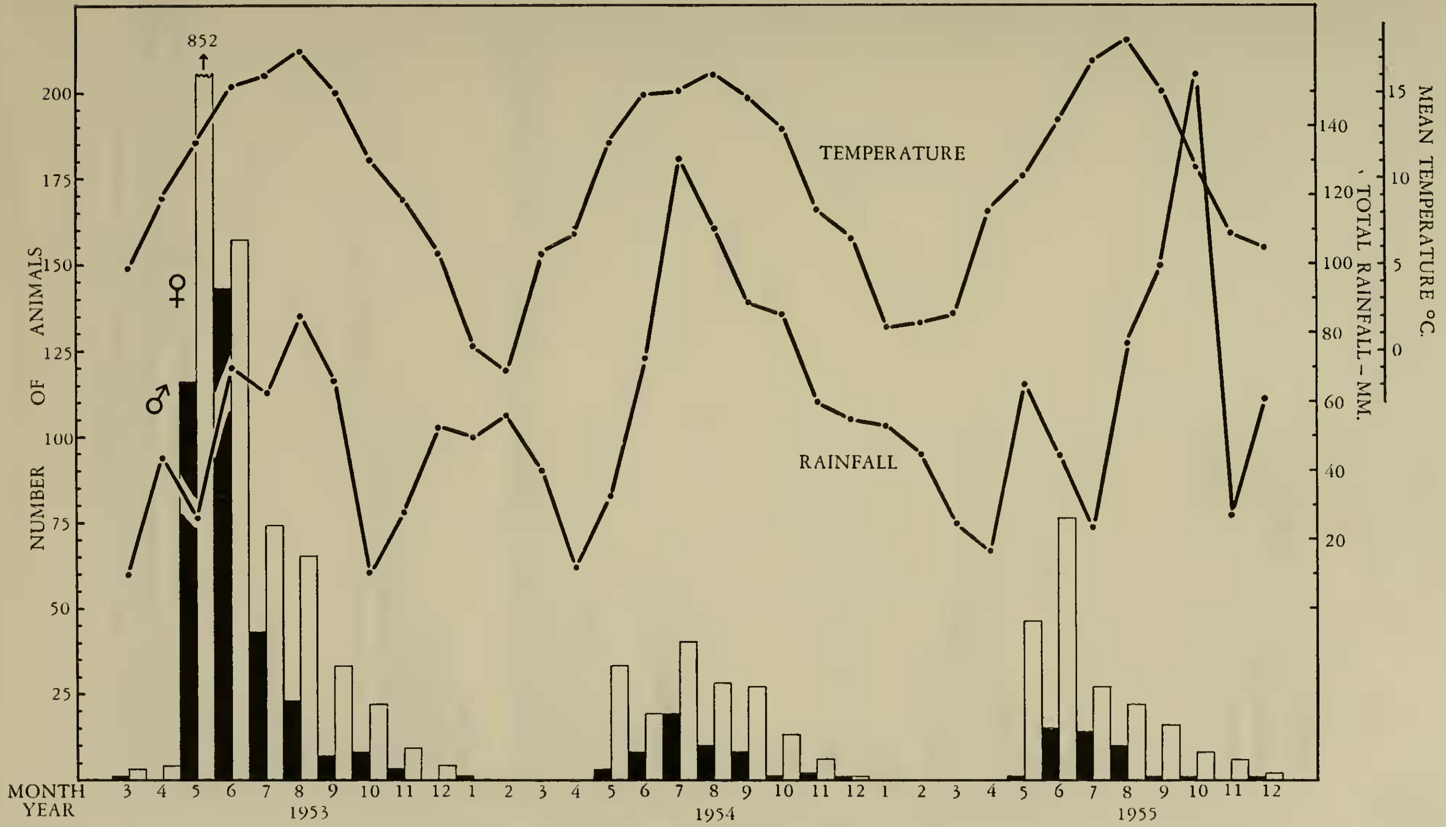


Fig. 6. Seasonal activity of *S. sabulosum* with mean monthly temperature and total precipitation per month. Columns represent total number of animals trapped in all habitats.

respect that the greatest interspecific difference in activity existed. *S. sabulosum* was active from May to December; the greatest number of animals of this species was trapped usually between May and August. *C. silvarum* and *I. scandinavius* were active generally from March to December although small numbers of *I. scandinavius* were trapped also during the winters. Both these species usually showed two activity peaks, one in spring and one in fall, although the autumn peaks of *I. scandinavius* were almost insignificant in 1954 and 1955. Between these periods of increased activity appeared a period of relative inactivity which extended over June and July with *C. silvarum* and usually from July to September in the case of *I. scandinavius*.

Somewhat similar phenomena have been observed in dry, hot countries where insects concentrate during the summer in certain habitats or enter some form of aestivation thereby avoiding excess heat and drought. A look at Figs. 4—6, however, reveals that, except in 1955, the greatest amount of rainfall was recorded between June and September. Thus it seems unlikely that the periods of summer inactivity of *I. scandinavius* and *C. silvarum* could have been due to existence of particularly dry conditions. Nevertheless, as will be shown later, all changes in the activities of the millipedes during the year are to some extent correlated with fluctuations in their behaviour towards humidity.

Figs. 4—6 show further that the mean monthly temperature was generally highest between June and September and lowest during January and February. Consequently, it seems feasible that activity minima in winter and mid-summer may be correlated with too low or too high temperature. This possibility will be further discussed when dealing with the influence of temperature and humidity combined.

Similar variations in the seasonal activity of ants were found by Bodenheimer & Klein (1930). They observed that activity during the year had maxima in April and December and minima in January—February and in August. Activity apparently was not related to rainfall but was greatly influenced by temperature. They measured the velocity of locomotion of the ants at different times of year and found that the minimum temperature at which activity began differed according to the season but without any definite relation to the outside temperature. This fact they considered to be indicative of seasonal variation in the physiological state of the ants.

It is also conceivable that seasonal changes in activity are influenced by reproduction. Unfortunately, too little is known about the reproduction of these millipedes to comment on this possibility.

Whatever the origin of the summer period of decreased activity, it is in some way different from overwintering since *S. sabulosum* showed no mid-summer inactivity, but all three species were largely dormant during the winter.

There was close agreement between the activity periods of the sexes of *I. scandinavius* and *C. silvarum* but some difference was exhibited by *S. sabulosum*. With this species, as a rule, relatively more females than males were trapped in May and the peak number of males was usually obtained about a month later than the peak number of females. Conversely, males of this species characteristically disappeared earlier in the fall than females which decreased more gradually in number. Overwintering is presumably initiated earlier and concluded later in males than in females. Further evidence of this phenomenon was shown by the reactions of the animals towards relative humidity.

SUMMARY AND CONCLUSIONS

From the distribution patterns obtained during the three years of analysis, the following points seem particularly conclusive.

Each species has a different type of distribution in the experimental area. *C. silvarum* is restricted entirely to a comparatively old terrain of birch and aspen woods. *I. scandinavius* has a much wider distribution than *C. silvarum* but is also characteristically a forest species. *S. sabulosum* occurs as well in open terrain as in woods and shows relatively little preference for particular habitats.

Females, particularly of the species *C. silvarum* and *S. sabulosum*, are slightly less restricted ecologically than males of the same species.

I. scandinavius and *C. silvarum* have characteristically two periods of peak activity during the year, one in early spring and one in autumn, which are separated by a period of relative inactivity in mid-summer. *S. sabulosum* becomes active in May, its peak activity occurring usually from May to August. All three species are more or less dormant during the winter.

Overwintering, at least in *S. sabulosum*, is commenced earlier and terminated later in males than in females.

With these facts in mind, the rest of the work is devoted to a comparative analysis of the reactions of the millipedes to the three environmental factors, humidity, temperature, and food. An attempt has been made to determine the influence of each factor on distribution and to explain as much as possible of observed differences between the distributions of the three species.

HUMIDITY

Climatic factors are influential in the distribution of animals through their effects on both behaviour and physiology. These two aspects are the foci of the following investigations designed to analyse the importance of humidity in the distributions of the three species of millipedes.

Changes in activity of millipedes during the year produce modifications, probably in their horizontal distribution, and certainly in their vertical distribution during overwintering. These changes in activity may be consequential both to differences in the water relations of their habitats during the year and to seasonal variations in their behaviour and physiological reactions towards humidity. Accordingly, it is also important to compare the reactions of species at different times of year.

HUMIDITY PREFERENCE

Many animals are able to perceive and orient to humidity differences in their environments. This orientation constitutes the behavioural influence of humidity on distribution.

METHOD.

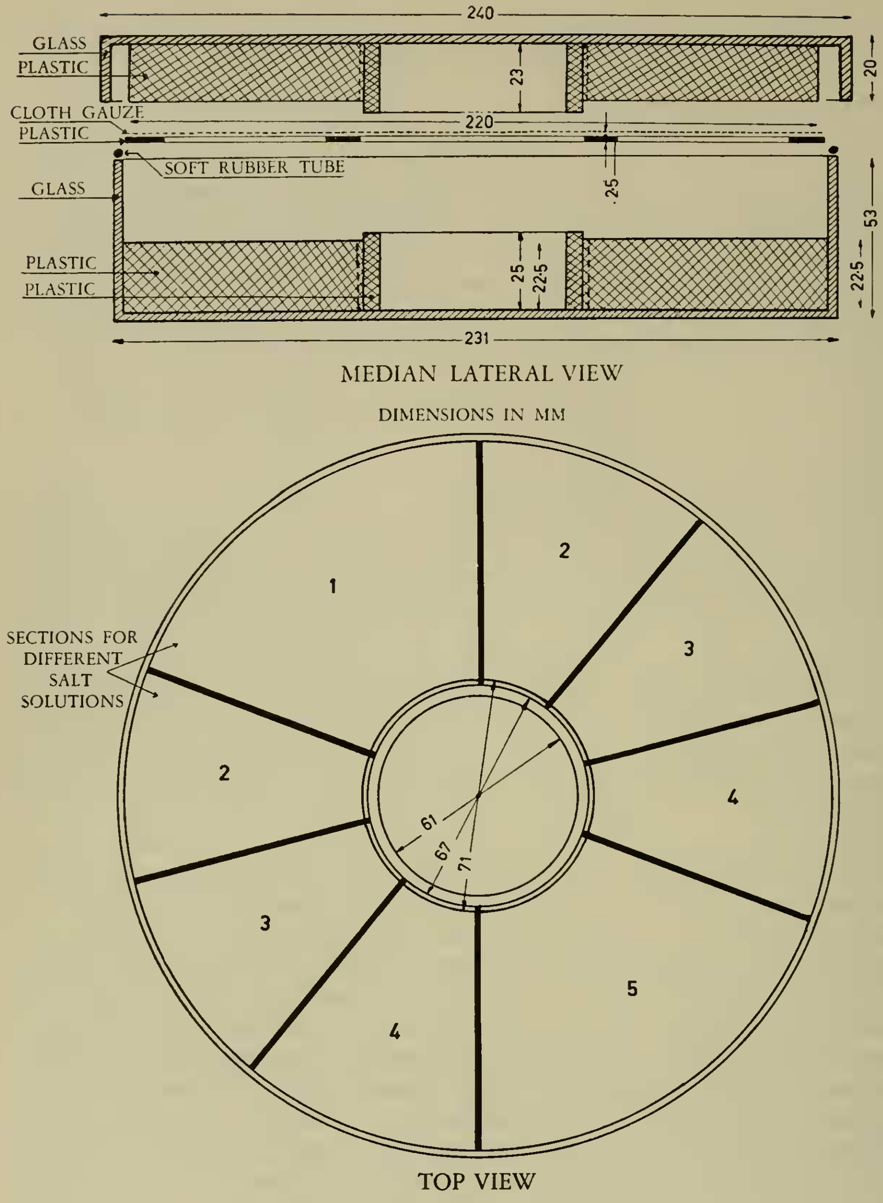
A humidity gradient apparatus (Fig. 7) was constructed from a large, glass, culture dish, the bottom half of which was radially divided with strips of plastic plate into eight sections. Each section was made watertight with plastic cement. Relative humidities were produced using saturated solutions of appropriate salts. Solutions were placed in the sections to produce two equal gradients of five relative humidities in each half of the dish. Because of this arrangement of the humidities, sections one and five, which produced the lowest and highest humidities, respectively, were made larger than each of the other sections by an amount necessary to produce equal experimental area at all humidities. The four salts used and the approximate relative humidities produced by their saturated aqueous solutions at a room temperature of 22° (PETERSON, 1947) were:

$Ca(NO_3)_2 \cdot 4H_2O$	— 55%
NH_4Cl	— 78%
KCl	— 85%
KNO_3	— 93%
Distilled H_2O	— 100%

A false bottom of cloth gauze stretched over a plastic frame and resting on the plastic partitions of the bottom of the dish served as runway for the experimental animals. The lid of the dish was divided similarly into eight sections by plastic plates. When the lid was in place, its sections corresponded vertically with the sections in the bottom half of the dish. The plastic partitions extended to about five mm from the false floor to reduce convection currents between adjacent sections of the gradient. The centre of the runway area, where the gradient was liable to be affected by close proximity of the solutions, was blocked off by a plastic cylinder. The lid of the dish rested on a narrow strip of soft, rubber tube glued to the rim of the dish and coated with vaseline which produced an airtight contact between lid and dish. Animals were introduced into the apparatus through a small hole in the lid of the dish. The hole was then plugged with a rubber stopper. The apparatus was prepared and left standing for 12 to 16 hours before an experiment to allow time for a stable gradient to be produced.

To begin an experiment, 10 animals were released into the lowest humidity of the apparatus. Experiments were carried out in darkness produced by enclosing the apparatus in a cardboard box. The positions of the animals in the apparatus were noted at 10 min., 15 min., ½ hour, one hour, and each hour thereafter for a total of seven hours. This method of observation biased the results slightly in favour of the early distribution of animals in the gradient. Because of this, the results indicate, to some extent, the speed with which a stable selection was reached, i.e., the intensity of selection. Individuals of all three species usually came to rest in the apparatus after about one hour, at the latest, but this time was often shorter depending on the intensity of attraction to a particular humidity. Animals to be tested were collected in Meijendel the previous day and kept overnight in a petri dish containing moist aspen leaves and placed in a cool, dark room.

During winter, *S. sabulosum* and *I. scandinavius* burrow into the ground and are difficult to find. To overcome this difficulty, wooden boxes were placed in the ground in a wooded area in Meijendel and stocked during summer with individuals of these two species. The boxes contained about 30 cm of soil with a



MEDIAN LATERAL VIEW

DIMENSIONS IN MM

TOP VIEW

Fig. 7. Humidity gradient apparatus.

layer of dead wood and leaves on top. The lids and bottoms of the boxes were of wire screen to permit normal conditions of light, temperature, and moisture. Experimental animals were obtained from these stocks during winter.

Selection of humidity by the three millipede species was examined at various times during the year. Each time, six tests were done with each sex of each species and the results of the six tests were averaged.

Results.

Interspecific and Intraspecific Difference

Fig. 8 represents the mean number of animals observed at each of the five humidities during all tests throughout the year. This is, therefore, a graph of average observations and since the selection changes during the year, as will be shown presently, the preferences of the three species may not necessarily be the same as these at any given time of year. But the figure serves to give an overall impression of the frequency with which individuals of a species were observed at each of the five humidities during the whole year.

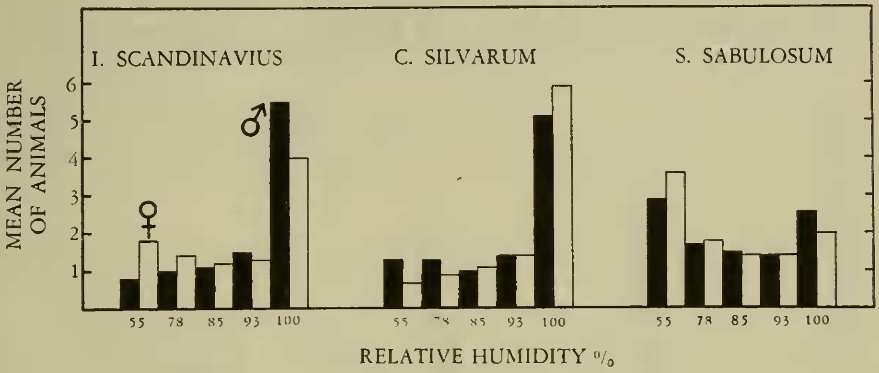


Fig. 8. Selection of humidity. Average results of 72 to 78 tests with each sex of each species throughout the year.

Differences between the selections of the three species are immediately obvious, primarily between *S. sabulosum* and the other two species. There was a prevailing preference for the highest humidity of the gradient on the part of *I. scandinavius* and *C. silvarum* while *S. sabulosum* generally showed equal preference for the two extreme humidities or a preference for the lowest humidity.

Orientation of the millipedes in the humidity gradient appeared to be largely orthokinetic. Changes in speed of locomotion were readily observable in animals passing from one humidity into another. After about an hour, experimental animals became completely motionless in the humidity of their choice after which they seldom moved again during the succeeding six hours. Although halts, turns, and testing movements typical of kineses and taxes, were displayed, these reactions were apparently not neccessary for maintenance of a selection once it became established.

There was little intraspecific difference in selection in the case of *C. silvarum.* With the other two species, however, *I. scandinavius* males showed a more pronounced preference for the highest humidity than the females and *S. sabulosum* females generally showed stronger attraction to the lowest humidity than males, which on the average did not discern between the two extreme humidities. This difference between the sexes can be seen more clearly by examining the selections at different times of year.

Seasonal Variation

Differences in humidity selection during the year were noted with all three species (Figs. 9 to 11). Each species selected humid air (100-%) during the winter months. With the advent of spring, however, this preference became less pronounced and, with *S. sabulosum*, shifted completely to selection of the driest air which, in these experiments, was 55% relative humidity. This preference of *S. sabulosum* for relatively dry conditions continued through the summer reaching maximum intensity in June through August during 1956. In September of that year, selection of the lowest humidity became less pronounced. This trend continued until a definite preference for the highest humidity was again exhibited in January, 1957. In 1955—56, this bi-annual shift in preference occurred earlier than in 1956—57. By October, 1955, conversion to selection of 100-% relative humidity was almost complete while, in October, 1956, the preference was still considerably more towards the dry end of the relative humidity range. Similarly, the shift in selection by *S. sabulosum* was completed earlier in spring, 1956 (March +) than during the following year (April +). Such temporal modification of the change of humidity preference may presumably be caused by variations in weather during comparable times in different years. What these variations may be is not known. It is interesting to note, however, that spring,

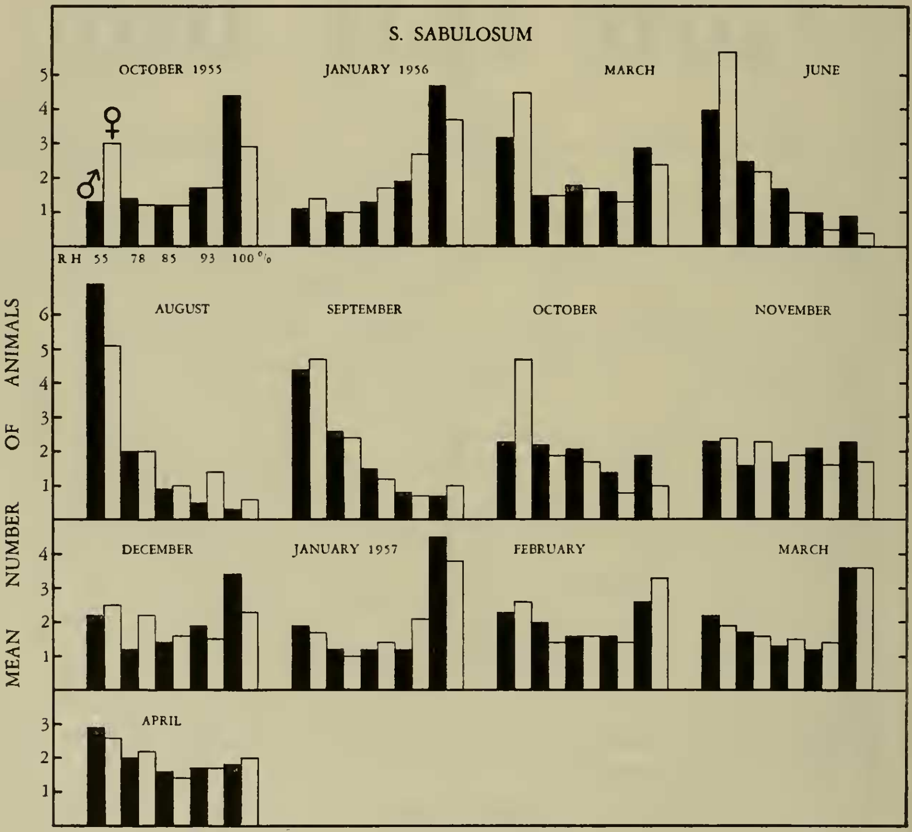


Fig. 9. Selection of humidity by *S. sabulosum*. Each figure represents the mean number of animals observed at each humidity during six, six-hour tests with 10 animals of each sex.

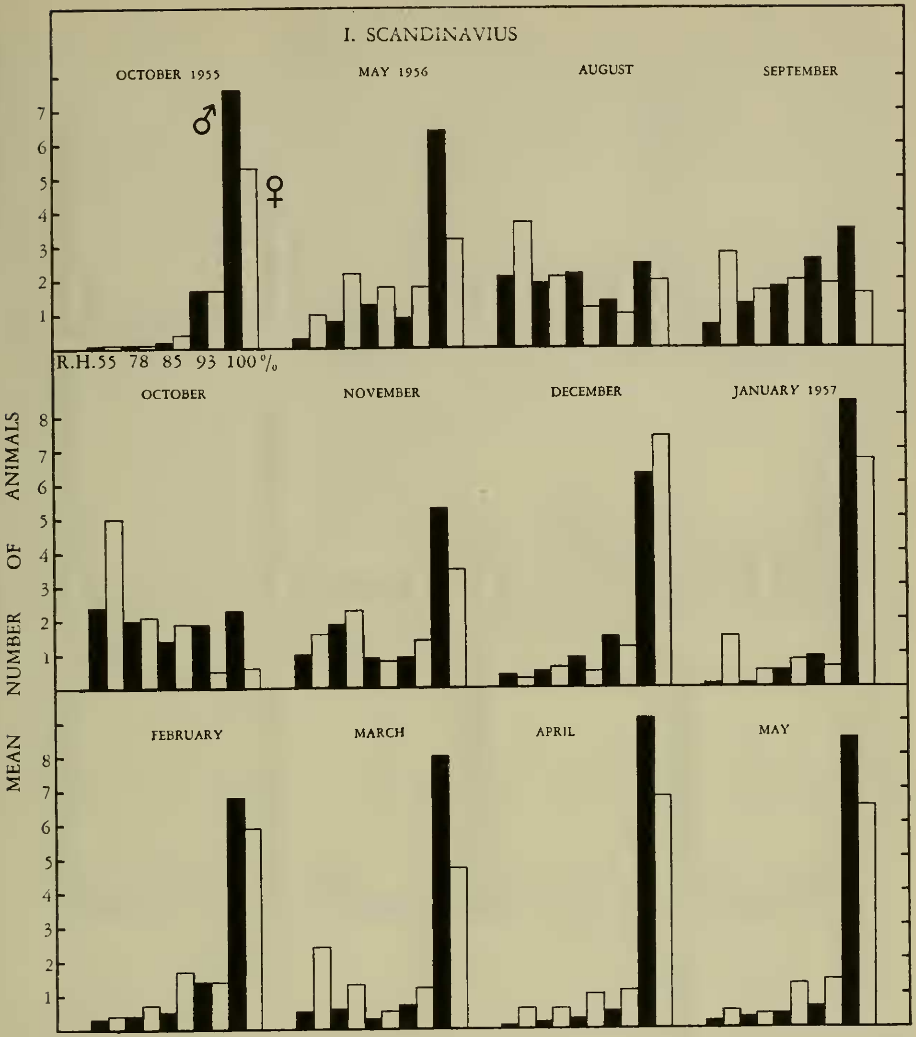


Fig. 10. Selection of humidity by *I. scandinavius*. Each figure represents the mean number of animals observed at each humidity during six, six-hour tests with 10 animals of each sex.

1957, was relatively dry. This condition may have produced some delay in the spring shift of relative humidity preference during that year. Subsequent evidence will show that the degree of activity of the millipedes in the field corresponds to existing conditions of temperature and precipitation.

Similar changes in humidity preference were observed with *I. scandinavius* although selection of low humidities by individuals of this species was never as pronounced as with *S. sabulosum*. But the basic pattern was similar, i.e., a relatively strong selection of the highest humidity during winter and spring which became less some time after May. With this species, the extreme shift towards preference of dry air was reached in October but had disappeared again by November when the highest humidity was again chosen.

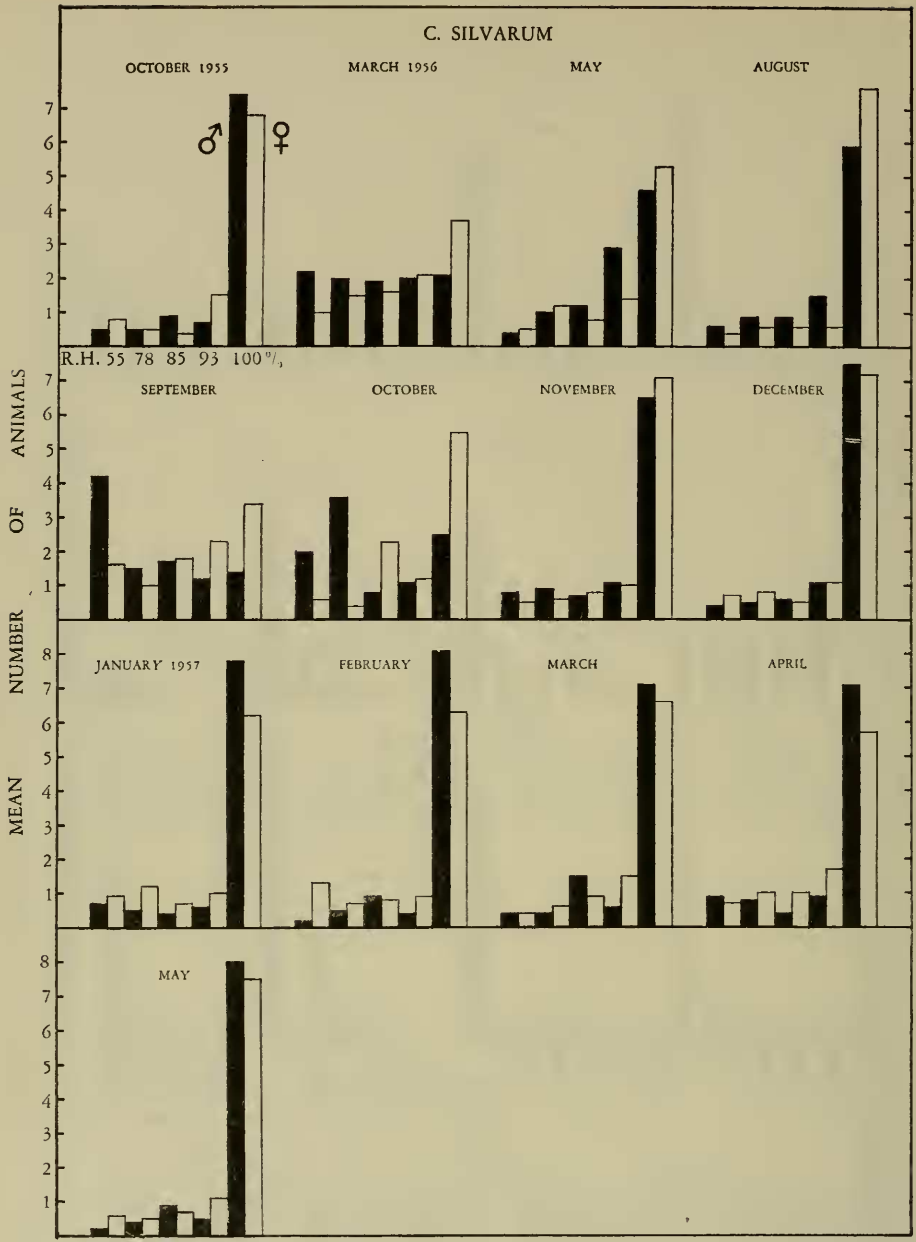


Fig. 11. Selection of humidity by *C. silvarum*. Each figure represents the mean number of animals observed at each humidity during six, six-hour tests with 10 animals of each sex.

Seasonal variations in humidity preference were less distinctly shown by *C. silvarum* but Fig. 11 suggests that selection of moist conditions by individuals of this species becomes less intense in early spring, in this case in March, but returns from May or earlier until September when a brief choice of dry conditions, or a lack of any preference, is again exhibited. By November, however, the preference in these experiments had again shifted back to 100-% relative humidity. During spring, 1957, no trend towards selection of lower humidities occurred; a strong preference for 100-% relative humidity was maintained. This lack of shift may have been caused by dry conditions which existed at this time as was suggested also as a possible explanation for the retardation of change in preference by *S. sabulosum* during the spring of 1957.

Some intraspecific difference in the course of seasonal changes also occurred. With *I. scandinavius* and *S. sabulosum,* the observed shift of selection towards 100-% in males almost invariably preceded that in females in autumn but lagged behind during the reverse reaction in spring. Males of these species also showed greater attraction to 100-% relative humidity (winter) and less attraction towards 55% (summer) than females of the same species. The reverse was sometimes true with *C. silvarum* males which, at times, showed less attraction to high humidity (March, 1956) or a more definite choice of low humidity (September—October, 1956) than females of this species.

Shift of humidity preference appeared to involve usually only the two extreme humidities in the gradient. There was no gradual shift during which successive humidities in the range were preferred. Determinations of how far selection of low humidities would extend during summer if the millipedes were presented with a complete range from 0% to 100% relative humidity and whether the difference between extreme humidities existing in the environment affects the shape of the selection diagram were not made but would yield valuable information.

DISCUSSION.

The experiments described above revealed four ecologically important points concerning the influence of humidity on the behaviour of the three species of millipedes. Primarily, all three species were able, normally, to orient in response to humidity differences in their environments. Secondly, intensity of reaction towards these differences varied among the three species. *I. scandinavius* and *C. silvarum* were usually strongly attracted to the highest humidity in the gradient but *S. sabulosum* showed greater independence of the humidity of the air.

The difference between reactions of *C. silvarum* and *S. sabulosum* agrees logically with what was stated previously about their distributions. *C. silvarum* typically inhabits wooded areas or areas with relatively dense vegetative cover. This floral canopy provides a comparatively humid environment by inhibiting evaporation of water in two ways. It decreases the amount of sunlight which penetrates to ground level. Air at this level is thus cooler, and consequently has less evaporating power than air in direct sunlight. Vegetative cover also restricts air currents thereby reducing the amount of water vapour which is conducted away. Considering the strong preference for high humidity usually exhibited by *C. silvarum* in the laboratory, it is not surprizing that this species is restricted to moist environments in nature.

The stenotopic existence of *C. silvarum* finds a counterpart in the behaviour of *S. sabulosum.* As Fig. 3 has shown, *S. sabulosum* is a typically eurytopic species. Besides inhabiting wooded areas, it also occupies many other types of terrain with apparently equal facility. Such a species is characterized by a certain plasticity towards environmental factors. This quality in *S. sabulosum* was shown by its relative independence of humidity differences and will be noted again when its reactions to other environmental factors are considered.

As a species which also has a wide range, *I. scandinavius* might be expected to show freedom from humidity differences like that displayed by *S. sabulosum.* Actually, in these experiments, it was generally strongly attracted to high humidity. A similar apparent contradiction will be noted also in reactions of this species towards temperature and will be discussed under a separate section.

A third important result of these experiments is the difference shown in intensity of selection of a certain humidity between the sexes of any one species. This difference is illustrated well by the selection histograms for any particular month. When high humidity was commonly selected, for instance during January, *I. scandinavius* and *S. sabulosum* males nearly always exhibited stronger preference for the highest humidity than females of the same species. During times when low humidity was selected, females of these two species showed stronger preference than males. This phenomenon suggests that males of these species are somewhat less free, ecologically, than females. Fig. 3 shows that this is actually true with *S. sabulosum,* females of which species have a slightly wider range than the males. But the distributions of male and female *I. scandinavius* (Fig. 2) are almost identical. Female *C. silvarum* also have a slightly wider distribution than the males (Fig. 1) although this fact was not corroborated by their humidity preferences. Closer correlations will be found between intraspecific differences in ecological freedom and resistance to water loss.

Certainly one of the most important points brought out by the results is that humidity preferences of these millipedes vary during the year. Modification of their behaviour in accordance with changes in their environments is obviously of great value in the survival of animals. Such variations in humidity preference could reasonably affect both the distributions of the species and their seasonal activities. However, little evidence was found of changes during the year in the actual habitats occupied by a species. Seasonal changes in humidity preference corresponded more closely with the numbers of animals which were active at different times of year. This is perhaps not odd if we remember that we are dealing here with macrohabitats and that we have no information about the "ecoclimates" (UVAROV, 1931) which exist in different microhabitats composing each macrohabitat which we are considering. Comparatively high humidities probably exist sporadically in nearly all types of terrain in this climate. The humidity under a few millimeters of soil, for instance, is undoubtedly higher than on the surface of the ground. Selection of high humidity by these millipedes during winter is, in fact, reflected in their burrowing into the ground, or into dead logs, where they overwinter and where their preference for moist conditions is supposedly observed. Thus it is possible that different habitats are occupied by these diplopods concordant with all changes in their humidity selections during the year. If so, these variations in habitat must be on a level not detectable by the method employed here.

Gradually increasing intensity of attraction to high humidity with the onset of winter apparently corresponds to diminishing activity in the field (Figs. 4—6). In winter, the animals burrow into the ground, or also into dead logs, as in the case of *C. silvarum,* perhaps partly in response to their preference for moister conditions. HODSON (1937) studied the moisture preferences of insects entering hibernation and found that most of the species preferred humid situations. A moist environment probably serves as protection during very cold weather since the latent heat of water tends to prevent a rapid subzero drop in temperature.

During spring, preference for moist conditions became increasingly weaker in *S. sabulosum* and gradually developed into a preference for drier air, which lasted throughout the summer. This reaction agrees well with the activity of this species which becomes maximum during summer.

The relation between humidity preference and activity of *I. scandinavius* was not as clear. In the year during which tests were made, this species showed a slightly decreased intensity of attraction towards high humidity in May after which the intensity decreased further until October. But according to results obtained during 1953—55 (Fig. 4), activity of *I. scandinavius* falls off during summer when its attraction to high humidity is supposedly less pronounced. It must be remembered, however, that seasonal activity during one year is being compared to humidity preference during another year and both these reactions are subject to variation.

C. silvarum has two periods of peak activity during the year, one in spring and one in fall. At these times, brief preferences for humidities below saturation or decreases in attraction to high humidity apparently occur. Changes in preference of this species, during the year, were less distinct and of shorter duration than those exhibited by *S. sabulosum* and *I. scandinavius*. Accordingly, *C. silvarum* lives constantly under more uniformly humid conditions.

Changes in the humidity preference of animals during the year have been mentioned previously by Perttunen (1951, 1952, 1953). He found the same reaction in *S. sabulosum* and suggested that the mechanism of shift in humidity preference was somehow connected with changes in ability of the animals to resist water loss. This possibility will be referred to again under consideration of the water relations of the millipedes. This subject constitutes what has been referred to as the physiological influence of humidity on distribution.

Resistance to Desiccation and Water Relations

The succeeding experiments were conducted to compare the abilities of the three species to withstand and restrict the loss of their body water when subjected to air of high evaporating power. Other water relations of the animals have also been determined in an attempt to learn as much as possible about the nature of water loss in these animals under drought conditions.

Method.

Saturated solutions of CH_3COOK were placed in glass preserve pots fitted with rubber rings and glass lids fastened down by metal clamps. The pots were kept in a thermostat at 22±°, at which temperature a relative humidity of about 22% was produced in each pot (Peterson, 1947). One animal was suspended above the solution in each pot by enclosing it in a perforated plastic box supported on a plastic coated wire tripod.

Fifty male and 50 female *S. sabulosum* and *C. silvarum* and 30 individuals of each sex of *I. scandinavius* were treated in this manner. All animals were weighed and placed in the pots as soon as possible after collection. They were examined for mortality each day, in the case of *S. sabulosum*, and every few hours, in the case of the other two species. The criterion of death was complete immobility when the animals were gently prodded with a fine brush. Each individual was weighed as soon as possible after it had died and then was desiccated for 48 hours at 105° and reweighed. The dry weight and initial water content were thus determined.

Essentially the same experiment was repeated at various times of year. During these tests, $Ca(NO_3)_2 \cdot 4H_2O$ was used to produce 55% instead of 22% relative humidity at 22±°. From 10 to 15 animals of each sex and each species were tested each time.

RESULTS.

Interspecific Differences

Particularly in tests with *S. sabulosum*, when experimental time was relatively long, loss of dry material by the animals during the experiment could, reasonably, have caused an error in calculation of the amount of water lost up to the time of death from the initial weight and the weight at death. BUXTON (1930) stated that if the mealworm, *Tenebrio*, is starved in dry air, it maintains a satisfactory proportion of water in its body by utilization of reserves and so produces and uses water of metabolism. Also HODSON (1937) reported that wasps exposed to low relative humidity at 6° had a smaller percentage dry matter after a certain time than individuals under the same humidity conditions but at 2°, i.e., there was an appreciable loss of material other than water.

As far as can be determined from the data of the present experiment, loss of dry weight due to utilization of reserves was negligible. No correlation was found between the proportion of initial weight lost before death and longevity. If starvation had any significant influence in weight loss, loss of weight due to consumption of reserves would likely be proportional to the length of the starvation period unless it is assumed that all reserves were used up in each individual before it died. If such was the case, it would be difficult to say whether water loss or starvation was the cause of death. Similar experiments throughout the year showed that loss of water definitely was a factor since longevity varied at different times although the relative humidity and temperature were always the same.

It is certainly only with *S. sabulosum* that an error in calculation of water loss could have been incurred by loss of dry material by experimental animals. Experimental time with the other two species was much too short to permit loss of a significant amount of dry matter. It is also only in calculation of percentage body water that loss of dry material during the experiment would be reflected. In determining percentage body water lost before death, weight loss up to the time of death was compared with the initial amount of body water and any loss of dry material during an experiment is contained in both these figures.

LONGEVITY. The mean longevities of the three species were all significantly different (Table I). By far the greatest longevity was exhibited by *S. sabulosum*. The longevities of the other two species were more nearly alike, but *I. scandinavius* lived slightly longer than *C. silvarum*. This contradicts, to some extent, the impression derived from similar experiments done throughout the year using 55% relative humidity. In these experiments, *C. silvarum* usually lived longer than

TABLE I. Longevity and water relations of millipedes at 55% R.H., 22±° C. Numbers are means and standard errors.

Species		Longevity — days	Water content %	% of initial water lost before death
I. scandinavius	♂	1.1 ± 0.04	69.1 ± 0.56	76.5 ± 1.21
	♀	1.4 ± 0.06	68.9 ± 0.93	73.0 ± 1.27
C. silvarum	♂	0.9 ± 0.05	58.0 ± 0.66	67.4 ± 1.88
	♀	0.9 ± 0.08	58.6 ± 1.16	63.8 ± 1.26
S. sabulosum	♂	15.8 ± 1.95	66.4 ± 0.58	68.9 ± 1.10
	♀	27.9 ± 2.52	68.6 ± 0.35	68.4 ± 1.04

I. scandinavius. It is possible that the time of year when each species was tested may have had some influence. The experiment with *C. silvarum* was done in August, at which time, individuals of the species showed strong preference for high humidity (Fig. 11). *I. scandinavius* were tested in October when they exhibited peak activity in the field and preference for low humidity, or lack of preference, in the laboratory.

Percentage body water. Of the three species, *C. silvarum* contained the lowest proportion of body water. The other two species were similar in this respect although *S. sabulosum* males had a slightly lower percentage body water than *I. scandinavius* individuals. The proportion of dry material to water content in the three species seems to have had little bearing on their resistance to drought. Although *C. silvarum* showed the lowest longevity and also possessed the lowest percentage water, figures for the other two species were not correlative.

Percentage water content lost before death. There was also little relation between the mean longevity of individuals of a species and the mean percentage initial water content lost before death. Figures for *S. sabulosum* and *C. silvarum* were similar although the mean for *S. sabulosum* females was significantly greater than that for *C. silvarum* females. *I. scandinavius* lost a greater percentage of their body water before death than the other two species.

Intraspecific Differences

Longevity. *I. scandinavius* and *S. sabulosum* females survived significantly longer than males of the same species. Similar experiments using 55% relative humidity also indicated that males are usually less resistant to the effects of drought than females of the same species. In the present experiment, male and female *C. silvarum* both exhibited the same longevity. It is possible that time of death was not determined accurately enough in this case to discern a difference between the longevities of males and females within the short experimental time.

Percentage body water. Only in the case of *S. sabulosum* was the percentage body water of the sexes different. Males had a significantly smaller proportion of water than females. The sexes of the other two species exhibited no significant difference in this respect.

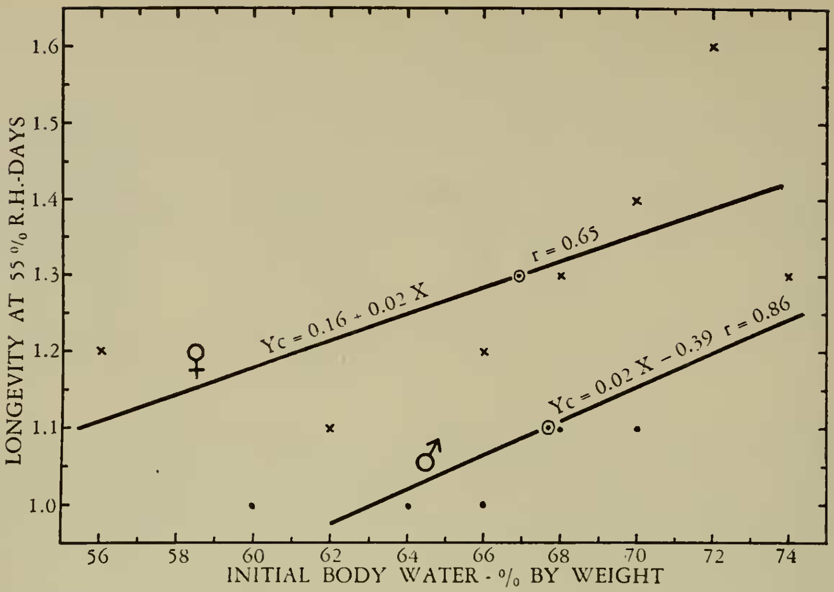


Fig. 12. Relation between longevity under drought conditions and percentage body water in *I. scandinavius*.

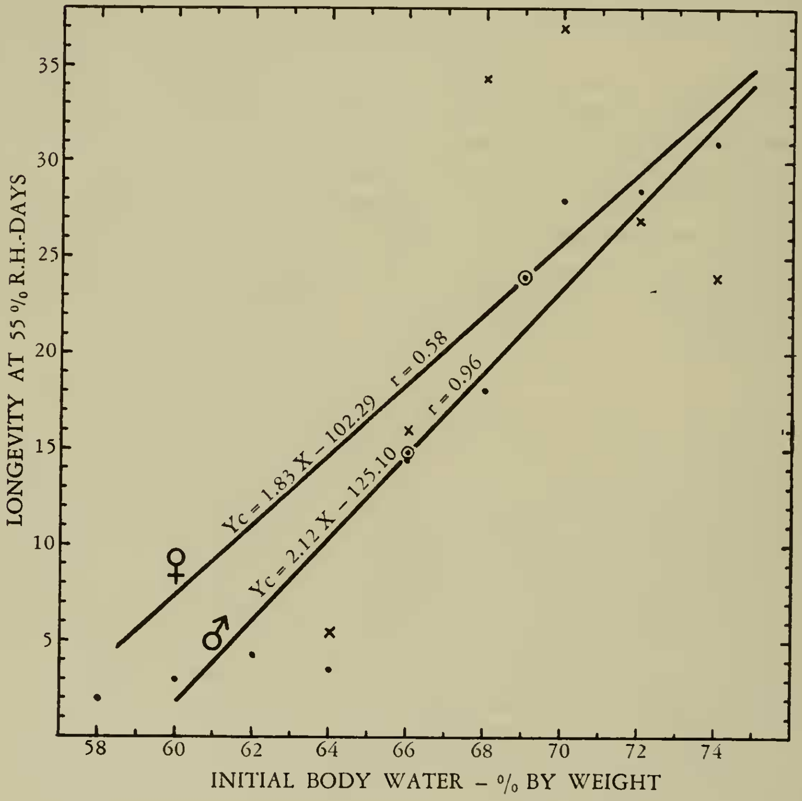


Fig. 13. Relation between longevity under drought conditions and percentage body water in *C. silvarum*.

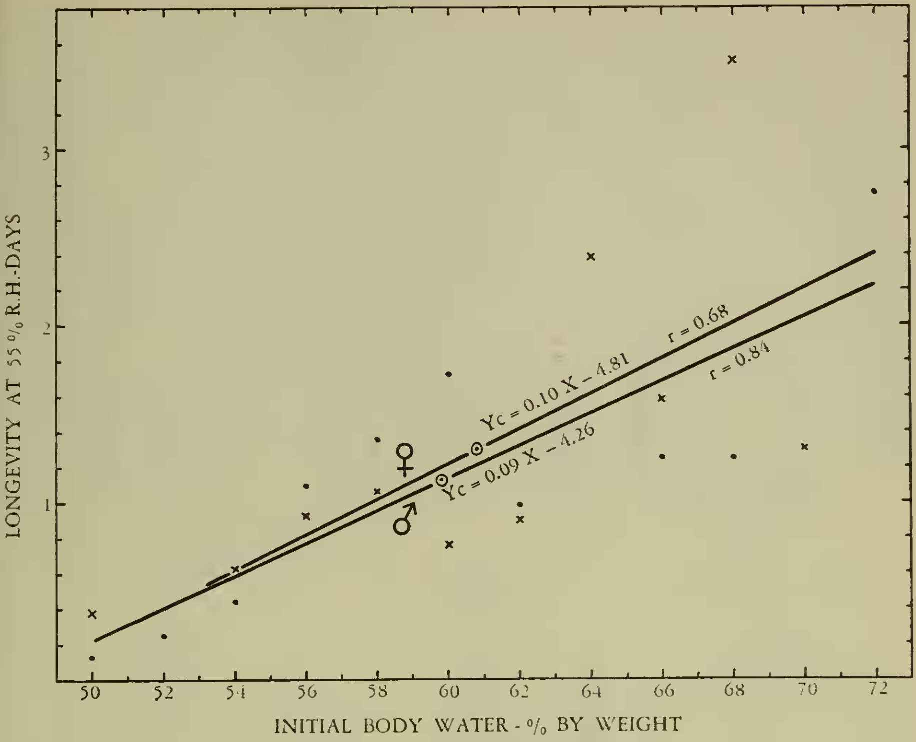


Fig. 14. Relation between longevity under drought conditions and percentage body water in *S. sabulosum*.

Percentage water content lost before death. *I. scandinavius* males lost a significantly greater percentage of their body water before death than females of the same species. There was a similar trend with *C. silvarum* although the difference was not statistically significant. The importance of this fact in relation to ability to withstand drought conditions is questionable since no correlation was found, within a species, between longevity and proportion of body water lost before death.

Data for all three species were analysed for correlations between several factors. The relations tested were: longevity and initial weight, longevity and percentage body water lost before death, percentage body water and initial weight, and longevity vs. percentage body water. Only with the last relationship were significant correlations found (Figs. 12—14). The functions are represented sufficiently well by straight lines although they appeared generally to be slightly curved.

Seasonal Variation

The mean longevities of individuals of each species kept at 55% relative humidity at different times of year are shown in Figs. 15—17 which illustrate a number of important points. With each species, there were definite times of year during which their resistance to water loss was superior to that at other times. The peak period of resistance in *I. scandinavius* occurred from August to October *C. silvarum* exhibited two maxima during the year, one in autumn and another, less pronounced, in early spring. *S. sabulosum* lived longest at 55% relative humidity during the summer months, from June through August.

Females of a species almost invariably lived longer than males of the same species. This fact further substantiated the superior resistance of females to water loss.

Seasonal changes in percentage body water are also shown in Figs. 15—17. In *I scandinavius,* percentage body water decreased from October to January after which it increased again. There was no decrease until January in *C. silvarum* and then only in females. The first decrease in percentage body water in males of this species occurred in March and it continued to fall in both sexes until April. Changes in this characteristic were not as clear in *S. sabulosum.* This could possibly have resulted from experimental error, as previously suggested, incurred in calculation of water loss by loss of dry material in animals of this species during the prolonged experiments.

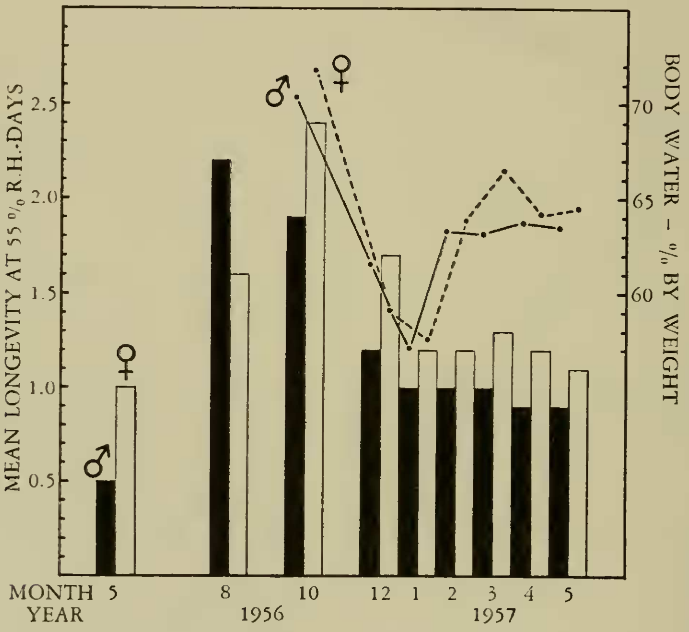


Fig. 15. Seasonal variation in resistance to desiccation and percentage body water in *I. scandinavius.*

No relation was found between changes in percentage water loss before death and the time of year. This fact attests to the lack of influence of this factor on survival at low humidity. The independence of resistance to desiccation and lethal

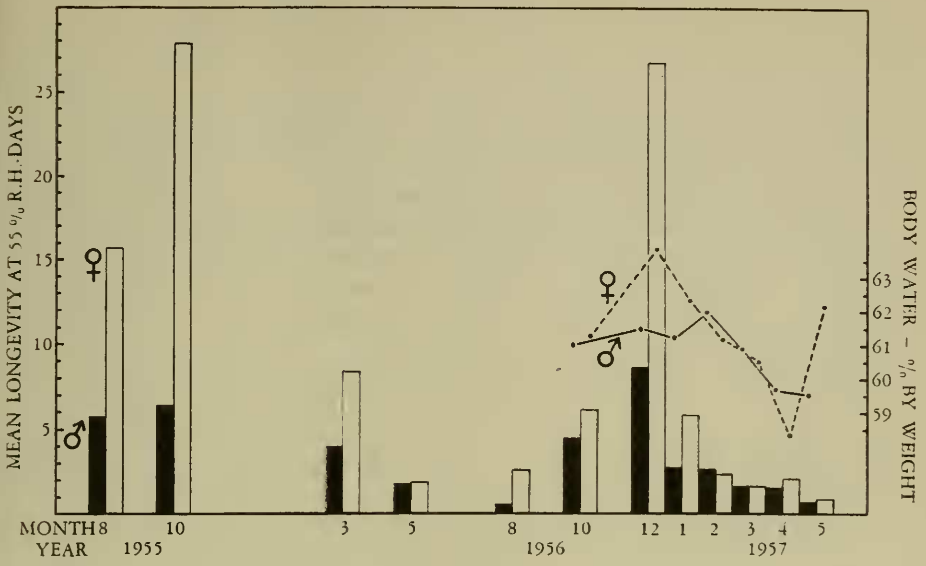


Fig. 16. Seasonal variation in resistance to desiccation and percentage body water in *C. silvarum*.

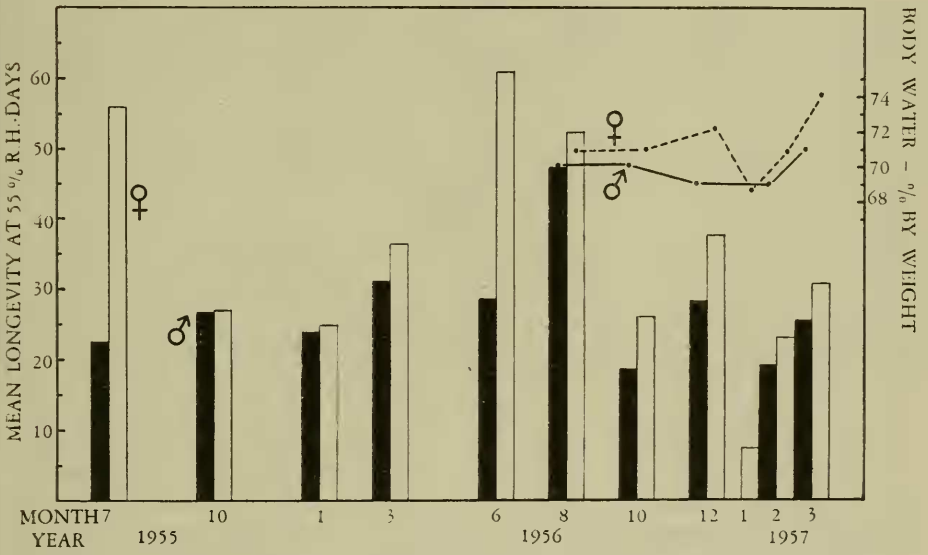


Fig. 17. Seasonal variation in resistance to desiccation and percentage body water in *S. sabulosum*.

limit of water loss can also be seen by examining Table II. In many tests of water loss at different times of year, there were, in each group of animals tested, one or two individuals which survived exceptionally long compared with the other animals of the group. Data for these long-lived individuals were not included in calculations for the group to which they belonged because their longevities were so far outside the distribution of longevities of the rest of the animals in the group. The water relations of these long-lived specimens are compared in Table II with data for "normal" animals tested at the same time. Here again, a connection between percentage body water and resistance to desiccation is indicated. Individuals which survived exceptionally long nearly always had a higher percentage body water than the mean calculated for other animals of the same group.

TABLE II. Body water relations of millipedes which survived exceptionally long at 55% R.H., 22±°, compared with those of other animals tested at the same time.

Date	Number of animals		Mean longevity — days		Mean % body water		Mean % initial water lost before death	
	♂	♀	♂	♀	♂	♀	♂	♀
I. scandinavius								
Oct., 1956		14		2.4 ± 0.11		71.8 ± 1.24		70.9 ± 1.92
		1		5.0		74.5		68.6
C. silvarum								
Oct., 1956	13	13	4.5 ± 0.83	6.2 ± 0.92	61.0 ± 0.97	61.2 ± 0.66	61.6 ± 1.52	56.0 ± 1.22
	2	2	34.0	57.5	68.1	69.7	64.5	67.0
Dec., 1956	14	13	8.7 ± 1.99	26.8 ± 4.88	61.5 ± 0.78	63.9 ± 1.35	64.1 ± 2.69	61.8 ± 1.79
	1	2	41.0	105.5	66.6	68.9	59.8	65.6
Jan., 1957	9	9	2.8 ± 0.43	5.9 ± 1.55	61.2 ± 0.54	62.4 ± 1.02	71.3 ± 3.84	65.3 ± 1.67
	1	1	27.0	42.0	66.0	67.4	60.3	69.0
Feb., 1957		14		2.4 ± 0.28		61.2 ± 0.55		64.4 ± 2.30
		1		14.0		61.1		50.7
Apr., 1957		14		2.1 ± 0.38		58.3 ± 0.67		57.5 ± 1.98
		1		43.0		68.0		65.7
S. sabulosum								
Oct., 1956		14		26.3 ± 5.90		71.0 ± 0.62		73.7 ± 1.90
		1		95.0		72.8		64.2
Dec., 1956		14		37.8 ± 5.31		72.2 ± 0.47		69.1 ± 1.51
		1		87.0		72.3		71.7
Apr., 1957	10	9	7.1 ± 1.42	5.6 ± 1.62	69.2 ± 0.53	72.8 ± 0.97	75.2 ± 3.66	68.7 ± 5.91

However, from the data, no relationship can be generalized between the exceptional longevities of the long-lived individuals and their lethal limits of desiccation. In only about half the cases was the percentage body water lost before death higher in the exceptional individuals than in the "normal" animals. Table II also illustrates the water relations of a number of *S. sabulosum* individuals tested in April, 1957. Nearly all of these animals were about to molt or had just molted and in this condition the cuticle is soft and pliable. These animals survived for a much shorter time than is usually expected of individuals of this species but their percentage loss of body water was, in fact, slightly greater than that generally observed with this species. In this case too, the short survival time was due to the rate at which water was lost and not to a decrease in the lethal limit of desiccation.

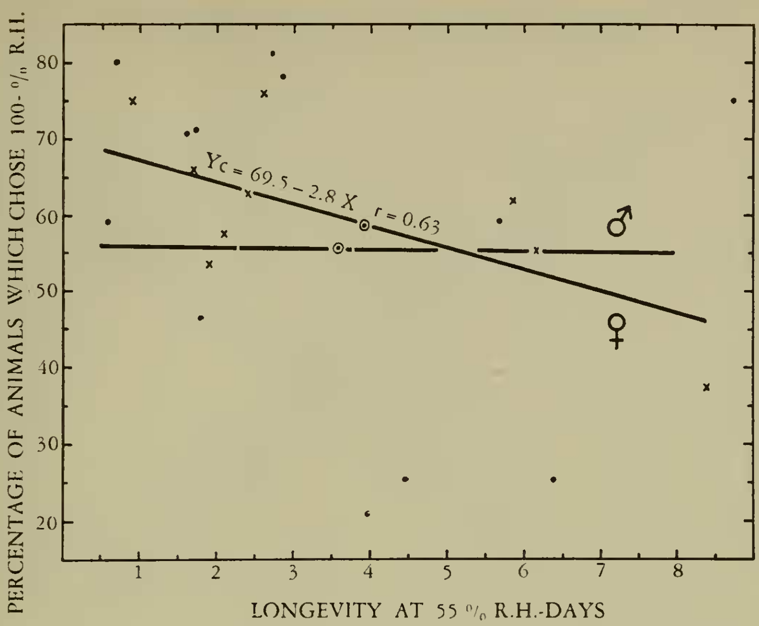


Fig. 18. Relation between intensity of attraction towards moist air and resistance to desiccation in *C. silvarum*.

Variations which were more or less in accord with the course of the year thus have appeared in resistance to water loss, percentage body water, and selection of relative humidity. To determine the relationships, if any, which exist between these factors, correlations between each pair were calculated. Figs. 18—20 show that, except with *C. silvarum* males, workable relationships existed between intensity of attraction towards 100% relative humidity and survival time at 55% relative humidity. Conversely, correlations were not found between percentage body water and either attraction to high humidity or longevity at 55% relative humidity. Although changes in percentage body water occurred during the year, these changes apparently were not influential in similar changes in resistance to water loss and selection of humidity. Correlations involving humidity preference were not outspoken with *C. silvarum* because this species nearly always exhibited an intense attraction towards high humidity and deviations from this reaction were indecisive and of relatively short duration.

DISCUSSION.

A number of rather complex interrelationships are apparently involved in the results of these experiments on humidity. It is perhaps best first to discuss the possible interpretation of these relationships and then to consider the results with respect to the distributions of the millipedes.

In a number of animals, resistance to desiccation has been found to be correlated with body size in accordance with the principle that surface increases by the square whereas volume increases by the cube so that larger individuals within a species survive longer under definite conditions of saturation deficiency because

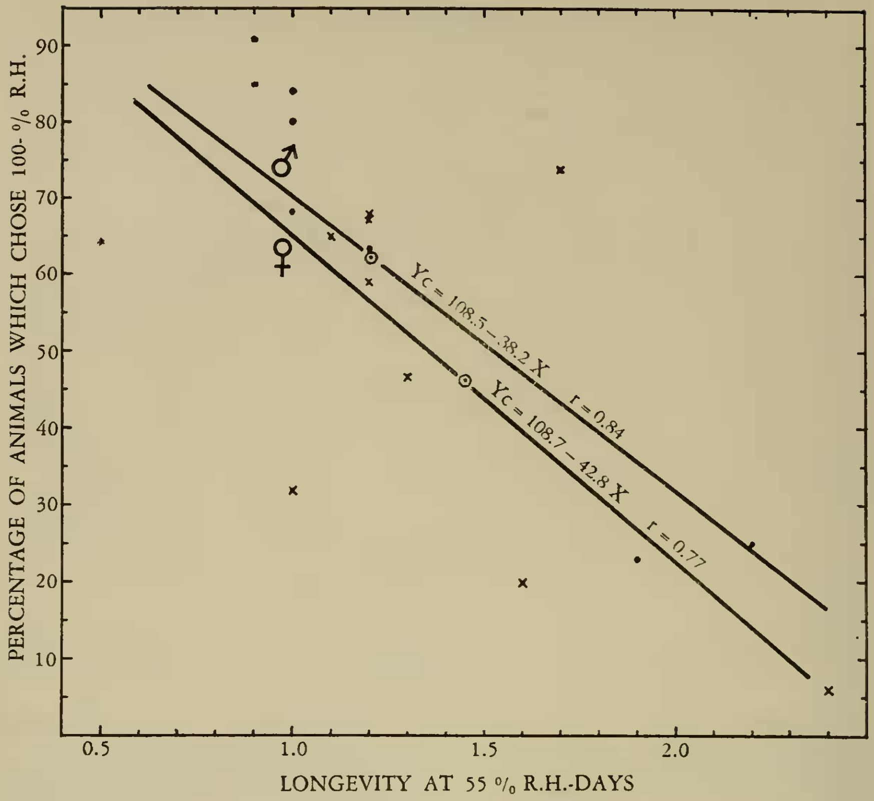


Fig. 19. Relation between intensity of attraction towards moist air and resistance to desiccation in *I. scandinavius*.

their evaporation surface per unit volume is relatively less than that of smaller individuals. Auerbach (1951) reported a positive correlation between the lengths of centipedes and their survival time under drought conditions. He maintained that this relation existed intraspecifically as well as between different species. A similar relation between weight and water loss in the millipede, *Paradesmus gracilis* (C. Koch), was claimed by Cloudsley-Thompson (1950a).

Although it was true, in the present experiment, that the larger, heavier, *S. sabulosum* survived longer than the other two smaller, lighter species, this relationship did not exist intraspecifically. There was no correlation between survival time and body weight within any of the three species. This fact provokes two conclusions. First, differences in longevity, both intraspecific and interspecific, of these millipedes under drought conditions, are not due to size differences between individuals. Differences in resistance to desiccation between species are due to specific characteristics and not to the general size factor. Second, the surface-volume principle is not applicable to the process of water loss by evaporation in these millipede species. In other words, water is not lost as in evaporation from an open surface. Loss of water must be more or less regulated by some structural mechanism or physiological process.

Ability of an animal to resist drought may conceivably be a function of its

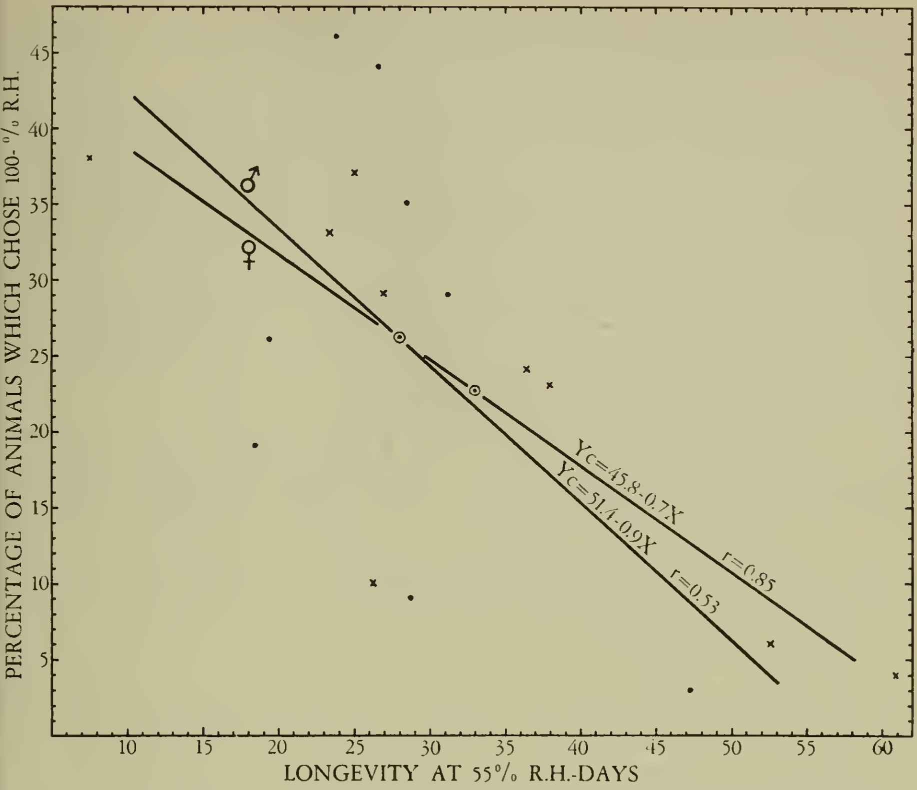


Fig. 20. Relation between intensity of attraction towards moist air and resistance to desiccation in *S. sabulosum*.

capability to withstand loss of a large proportion of its body water. This possibility was not substantiated by the present experiments. There was no correlation between longevity and amount of water loss which was tolerated. The percentage body water which was lost before death was a relatively constant quantity among the individuals of a species and the interspecific variation in this value was obviously not sufficient to explain the observed longevity differences. Further, although resistance to water loss varied during the year, no correlative seasonal variation was found in the lethal limit of desiccation. Animals which survived a shorter period of time at low humidity lost roughly the same amount of water before death as animals which lived longer, but they apparently lost their water faster.

Intraspecifically, correlations were found between longevity of animals under conditions of drought and their percentage body water. This factor may conceivably be of influence in the ability of animals to withstand low humidity. There are a number of facts, however, which indicate that the factor, body water content, is not sufficient to explain this ability. Initially, interspecific differences in longevity of animals maintained at 22% relative humidity were too great to be attributed solely to differences which existed in the proportion of water contained in the bodies of individuals of different species. Also, the survival times of the few individuals which were exceptionally long-lived, in tests at 55% relative humidity, were much too different from the mean longevities, calculated for other individuals tested simultaneously, to be accounted for by their slightly greater water contents. And lastly, while changes in percentage body water occurred during the year in each species, these variations were not sufficiently in accord with concurrent changes in resistance to water loss to explain that characteristic. Although the cause of correlation between percentage body water and resistance to desiccation is not known, it is fairly clear from these experiments that neither the initial proportion of body water nor the percentage loss of water before death can explain the ability of these millipedes to withstand drought. Differences between resistances of individuals is dependent on their ability to restrict water loss and not upon their limits of tolerance. This same conclusion was reached by HODSON (1937) while studying the role of water in insect hibernation.

It seems likely that the seat of resistance to desiccation lies, to a greater or lesser extent, in the cuticular layers of the body. This could explain the specific character of drought resistance in these animals. Detailed studies of arthropod cuticles (BEAMENT, 1948; WIGGLESWORTH, 1948) have indicated clearly that the site of resistance to transpiration in arthropods lies in this structure. This restricting character of the cuticle appears generally to be attendant on the presence of one or more lipoid layers plus sclerotization. It is not clear, however, what the restricting property of the cuticle is in this case or how it works. Freshly molted *I. scandinavius*, on which the new cuticle had not yet hardened, have been observed to be as resistant to water loss as specimens with hardened cuticle. Similarly BUXTON (1930), while testing the evaporation of water from mealworms at low relative humidity, noticed that several specimens which had molted during an experiment, did not lose weight. CLOUDSLEY-THOMPSON (1950a) also noted no significant difference between water loss from recently molted, pale, *P. gracilis*, and more mature, darker ones. These cases suggest that any property of the cuticle responsible for restriction of water loss may be independent of hardening of the cuticle. Conversely, many of the *S. sabulosum* tested in April, 1957, had recently molted or were about to molt and these specimens lost water more rapidly than individuals which were not in molting condition. In the above cases, however, it is not certain to what stage development of the new cuticle had progressed.

In an examination of the cuticle of diplopods, CLOUDSLEY-THOMPSON (l.c.) described hardening of the cuticle as due to both calcification of the outer endocuticle and tanning of the exocuticle. Studying the relation between water loss from *P. gracilis* and temperature, this same author found no evidence of a critical temperature and thus of a discrete wax layer to restrict transpiration. He also ascertained that experimental abrasion of the millipedes did not increase their rate of water loss and this also indicates the absence of an epicuticular wax layer. *P. gracilis* is a species which is very sensitive to desiccation and, although the cuticle possesses a certain degree of impermeability, the animals lose weight even over distilled water.

It is obvious that we are concerned here with three species of millipedes which are different from each other and from *P. gracilis* in their abilities to restrict water loss. The cuticle, certainly, is the site of resistance to desiccation but where this property is localized within the cuticle and how it differs in various species remains obscure.

Referring to the proportion of dry material to water in the bodies of insects, BUXTON (1932) wrote that, as a general principle, the larger the organism, the greater is the proportion of skeleton, for mechanical reasons. The proportion of water in the organism as a whole must therefore fall with increasing size. TEISSIER (1931) stated that every time the mealworm doubles its weight its dry material is increased 2.085 times, its water 1.965 times. Similarly, according to BODINE (1921), the larvae of a certain species of grasshopper contain 77.6% water, the adults about 72%. If adults are considered, the water content falls consistently with rising weight. BODINE thought this decrease of water was due to increasing age, per se, and not to any mechanical reason. Considering these facts, it is interesting to note the lack of correlation between the weights of millipedes in the present experiment and the proportion of water to dry material in their bodies. Interspecifically, *C. silvarum*, the smallest of the three species, contained the smallest proportion of water. The other two species were similar. Intraspecifically, the same absence of correlation between body weight and proportion of dry matter to water was apparent. The standard errors of the mean values of these two characteristics showed little variation within a particular species. The proportion of dry matter to water in these animals seems to be a specific peculiarity which is not influenced by the total body weights of the animals.

Interspecific differences in resistance to water loss in these millipedes seem to depend on differences in the degree of impermeability of the cuticle to water rather than on capacity to withstand loss of a large amount of water. In each species, ability to survive drought conditions varied at different times of year. These seasonal fluctuations in drought resistance are, likewise, not ascribable directly to the water relations of the animals but apparently result from changes which take place in the permeability of the cuticle. What causes these changes is not clear. In *S. sabulosum* and *I. scandinavius,* two changes per year occur, one in autumn and one in late spring, or late summer in the case of *I. scandinavius*. But with *C. silvarum* four changes per year must occur since individuals of this species have two periods when they are resistant and two periods when they are particularly susceptible to water loss. These changes, then, are cyclic and of a definitely specific character. If these variations are due to seasonal fluctuations in climatic conditions, these conditions must influence each species differently since the states of resistance of the species correspond only during the winter.

Variations in humidity preference also occurred and were correlated with the changes in resistance to water loss. The obvious conclusion entailed in this correlation is that these two phenomena are interdependent and perhaps, ultimately, have a common cause. That cause might logically be change in permeability of the cuticle. This idea was mentioned by PERTTUNEN (1953) as a possible explanation for agreement which he also found between resistance to desiccation and humidity preference in *S. sabulosum*. He assumed that rate of water loss was important in determining the direction and intensity of the humidity reaction although he emphasized that other factors must also be involved. Rate of water loss is not likely the mechanism directly responsible for change in the intensity with which an animal is attracted to a certain humidity since the animal probably has little realization of the rate, per se, at which it loses water.

Intensity of selection is more likely dependent on physiological changes brought about by loss of water. PERTTUNEN (l.c.) showed that the "dry reaction", exhibited by *S. sabulosum* individuals in summer, could be reversed by subjecting the animals to desiccation. Loss of a certain percentage of their body water resulted in a shift in their preference towards higher humidity. In the present experiments, differences were noted in percentage body water of individuals of all three species tested at various times of year and these differences were more or less correlative with seasonal changes in intensity of attraction towards high humidity and resistance to water loss. Considering PERTTUNEN's results, seasonal changes in body water level may conceivably be influential in simultaneous changes in humidity preference. But present data are inadequate to prove this theory. Substantial correlation between changes during the year in percentage body water and humidity preference was not obtained. The three factors, humidity selection, resistance to water loss, and body water content, definitely seem to be interrelated but more work is needed, particularly with individual animals, to clarify these relationships.

Besides the possible influence of body water content on other reactions, the seasonal change in this characteristic itself is undoubtedly an important factor in survival during winter. PAYNE (1926) stated that the ability of animals to resist low temperatures was associated with low body water content and ROBINSON (1927) showed that, in cold-hardy forms, the hydrophilic colloids of the body take up water on exposure to low temperature thus reducing the amount of freezable water in the insect. SACHAROV (1930) described cold hardiness in insects as depending "on the minimum quantity of water and the accumulation of fat in the organism. All hibernating stages of insects prepare themselves to withstand cold by reducing the quantity of free, easily freezable water in their tissues." In this connection, it is interesting to note that seasonal change in percentage body water was most marked in *I. scandinavius* and that this species was the only one of the three which was regularly trapped during the winter.

Overwintering in these millipedes is not a diapause in the sense of an obligate, partial arrestation of life processes. It is a purely facultative reaction; at least, a normal level of observable activity is resumed by animals which are brought into the laboratory during winter. Although PERTTUNEN (1953) mentioned that increase in intensity of attraction to high humidity was observable in animals previously kept for considerable time in the laboratory, it is unlikely that these animals would exhibit the burrowing reaction typical of overwintering millipedes in nature.

Selection of high relative humidity is obviously an asset in overwintering but it cannot be the primary cause of the vertical migration typical of this process in diplopods. Both *I. scandinavius* and *C. silvarum* show strong intensity of attraction to high humidity at times during the normal period of activity although this preference for moist conditions does not result in burrowing by the animals during these times. Other factors must be involved in initiation and termination of overwintering. In a number of cases (BREITENBRECHER, 1918; TOWNSEND, 1926;

Hodson, 1937) return to normal activity of various hibernating insects involves gain in water under the influence of changes in environmental temperature. Some such reaction, dependent upon the environmental complex, may be responsible for the overwintering process as it occurs in these millipedes. The pattern of loss and gain of body water during winter and spring agrees particularly well with this theory.

The distributions of *C. silvarum* and *S. sabulosum* correspond closely to their resistances to water loss. At humidities below saturation, *C. silvarum* loses water relatively rapidly. This species, accordingly, is found in a limited area consisting of wooded terrain with dense floral undergrowth where comparatively humid surroundings exist. Conversely, *S. sabulosum* loses water very slowly in dry air and is consequently able to exist in a greater variety of habitats. *I. scandinavius* is equally or more susceptible to water loss than *C. silvarum* but nevertheless is found under dry as well as humid conditions. Similar inconsistency between selection of humidity and distribution of this species has already been noted and will be treated in a subsequent section.

Differences in ability to withstand desiccation exist also between the sexes, particularly of *C. silvarum* and *S. sabulosum*. Males of these species characteristically lose water more rapidly than females and this difference is reflected in their distributions. Females of both species have slightly wider distributions than males of the same species. Little difference was discernible between resistance to desiccation of male and female *I. scandinavius*. Both sexes lost water very rapidly. The distribution patterns of this species, accordingly, show no intraspecific difference.

Variations during the year in ability to resist desiccation generally corresponded well with the periods of activity of the millipedes. All three species are relatively inactive during the winter and, at this time, were least resistant to desiccation. *C. silvarum* individuals showed a slight increase in resistance in early spring which corresponded to their increased activity during this time. Their resistance then decreased during the summer after which it again increased to a peak in the fall when the animals became most active. Although *I. scandinavius* typically exhibited greatest activity during spring, no increase in resistance to desiccation was revealed in tests with this species during that time. Individuals kept under dry conditions lived longest between August and October. *S. sabulosum* individuals were most resistant to water loss from June to August during which months they also exhibited peak activity. Males of this species became most resistant slightly later in the summer than females. Their activity, correspondingly, reached its peak about a month later than the activity peak of the females.

Selection of humidity by the three species was correlated, both interspecifically and within any one species at different times of year, with their ability to restrict water loss. In animals such as millipedes, which tend to lose water at all humidities below saturation, the realization of and reaction to humidity differences in their environments is constantly necessary and has obvious survival value in preventing the animals from being "trapped" in areas where they might perish from loss of large amounts of water.

In conclusion, humidity is unquestionably an important limiting factor in the distributions of these species. Its influence is revealed both by the behaviour and by the physiological reactions of the animals. Great differences exist between the abilities of the species to withstand dry conditions and the characteristic orientation of individuals of a species towards humidity differences in their environment depends on this capacity for water retention. Reactions of these diplopods towards humidity, except in the case of *I. scandinavius*, agree closely with their distributions in the field and, without exception, appear largely to reflect the extent of their activities in nature.

SUMMARY

The following points represent a concise account of the relationships revealed by the foregoing experiments.

All three species of millipedes exhibited ability to orient in response to humidity differences in their environments.

Interspecific differences in intensity of attraction to high humidity were displayed which were correlated with differences between the abilities of individuals of the species to resist water loss under drought conditions. Selection of humidity by these millipedes thus acts as a protective mechanism leading the animals to conditions suitable for their water conserving properties.

Humidity reactions of *C. silvarum* and *S. sabulosum* conformed closely to their distributions in nature. *C. silvarum* leads a stenotopic existence, under natural conditions, and correspondingly was strongly attracted to high humidity and showed little resistance to water loss. *S. sabulosum,* which has a wide and varied distribution, oriented less intensely to humidity differences in its environment and exhibited strong resistance to desiccation. Reactions of *I. scandinavius* to humidity did not agree with its observed distribution. Individuals of this species were strongly attracted to high humidity and proved very susceptible to water loss although their distribution is almost as widespread as that of *S. sabulosum.*

Males of the species *C. silvarum* and *S. sabulosum* showed stronger preference for high humidity and slightly less resistance to desiccation than females of the same species. This difference allows a somewhat greater ecological freedom in females than males and this can be seen in their slightly wider distributions.

In each species, cyclic changes in ability to resist desiccation occurred during the year and were generally correlated with contemporary shifts which usually took place in humidity selection. These changes are apparently, to some extent, responsible for variations in activity during the year.

Decreases were noted in the body water contents of these millipedes during late fall and winter and may serve as protection against damage of the tissues due to freezing. Fluctuations in body water level may also influence the initiation and termination of winter dormancy.

The ratio of dry matter to water in the bodies of these animals was found to be a specific characteristic which is not correlated with body weight.

Resistance to water loss was not influenced either interspecifically or intraspecifically by body size. However, it was correlated, within any one species, with the proportion of dry matter to water in the body but this characteristic did not satisfactorily explain either the difference between abilities of different species to withstand drought conditions or similar differences between individuals of any one species.

Ability to endure desiccation was revealed to be a function of restriction of water loss rather than the lethal limit of desiccation. The cuticle is the site of restriction of evaporation but the mechanism responsible for this control, and the changes which must take place in permeability of the cuticle during the year, are not known.

TEMPERATURE

KROGH (1914) suggested that the influence of temperature as a habitat factor is twofold: a relatively prompt effect mediated by the central nervous system, and a slower effect through the alteration of general metabolism. Both types of effect must be considered in order to understand the characteristic influence of temperature as a factor in the ecology of an animal species.

TEMPERATURE PREFERENCE

The immediate reactions of animals towards unfavourable temperature constitute "searching" movements responsible for reaching and maintaining a situation in which a preferred, and supposedly more suitable, range of temperature exists. Since preferred temperatures may vary considerably between different species, their determination may be important in a comparative analysis of temperature as a factor in the distribution of the species.

Temperature is basic also to seasonal variations in behaviour (ALLEE et al., 1949) and thus distribution, making periodic examination of temperature preference throughout the year of value too.

METHOD.

The preferred temperatures of the three species of millipedes were determined at various times during the year in conjunction with the experiments on relative humidity preference. Tests were done using a circular temperature gradient apparatus, described by BARLOW and KUENEN (1957), in which animals were offered a choice of nine temperatures between approximately 5° and 30°, produced in 16 semi-isolated cells. This construction made possible the maintenance of air humidity near 100% at each temperature thus decreasing the effect of an inverse gradient of relative humidity.

To begin an experiment, one specimen was released into each of the 16 cells of the apparatus. The distribution of the animals and the temperature of the air in each cell were noted every hour for six hours. During this time, the ice at the cool side of the apparatus was renewed twice and the temperature of the water at the warm side adjusted to compensate for changes in room temperature. At the end of six hours, the mean of the temperatures recorded for each pair of corresponding cells on each side of the apparatus was calculated. The numbers of animals observed in each pair of cells each hour were also averaged. With this method, nine mean temperatures were obtained with the mean number of animals which was observed at each of the nine temperatures. These figures were averaged for all experiments in each test series. All experiments were conducted with the cells of the apparatus darkened.

Animals to be tested were collected the previous day and kept overnight in a large petri dish containing moist, rotten, aspen leaves, which all three species eat readily, and placed in a cool, dark, room.

For purposes of comparison of results, preferendum was taken to signify the mode of the distribution of animals in the apparatus. When, as usually happened, the mean numbers of animals observed at a series of temperatures were alike statistically but significantly greater than the mean number observed at any other temperature, the preference was considered to include the whole series of temperatures. For example, in tests with *I. scandinavius* males (Fig. 21), the animals chose temperatures of 16.3° and 19.2° equally as often but with significantly greater frequency than any of the other temperatures. Thus, in this case, the preferred temperature was taken as 16.3° to 19.2°. In examining data, the total range of temperature over which animals were observed, the preferred range, and the number of animals in the preferred range, i.e., the intensity of the selection, were noted.

RESULTS.

Interspecific and Intraspecific Difference

The combined results of all tests are represented in Fig. 21. Differences between the preferred temperatures and between the intensities of selection are immediately apparent. The highest temperatures (16.2° to 19.2°) were chosen by *I. scandinavius,* while *C. silvarum* chose temperatures between 9.3° and 16.5°. In general, the distribution of *S. sabulosum* in the apparatus showed no definite preference and indicated only a tendency to avoid the highest temperatures in the range.

I. scandinavius preferred not only the highest temperatures of the three species, but also exhibited the highest intensity of preference. The range of preference extended over only 3° (16.2° to 19.2°) while *C. silvarum* preferred temperatures in a 6° range. The total range of distribution of *I. scandinavius* in the apparatus was also narrower than that of the other two species. The lowest intensity of preference was shown by *S. sabulosum* which were regularly observed over the whole range of temperatures.

With each of the species, the preference of males was very similar to that of females.

The mechanism of temperature preference appeared to be more complex than that of humidity preference. Besides orthokinesis, the halts, turns, and testing movements typical of taxes were more often observed. The maintenance of a temperature preference was apparently more actively achieved; millipedes in a gradient of temperatures became completely motionless more often than in a gradient of humidities.

Seasonal Variation

Figs. 22—38 summarize the results of a number of series of tests with each species at different times during the year. No obvious and regular change in the temperatures selected, such as that observed in the humidity preferenda of *S. sabulosum* and *I. scandinavius*, appeared. There was, however, a certain amount

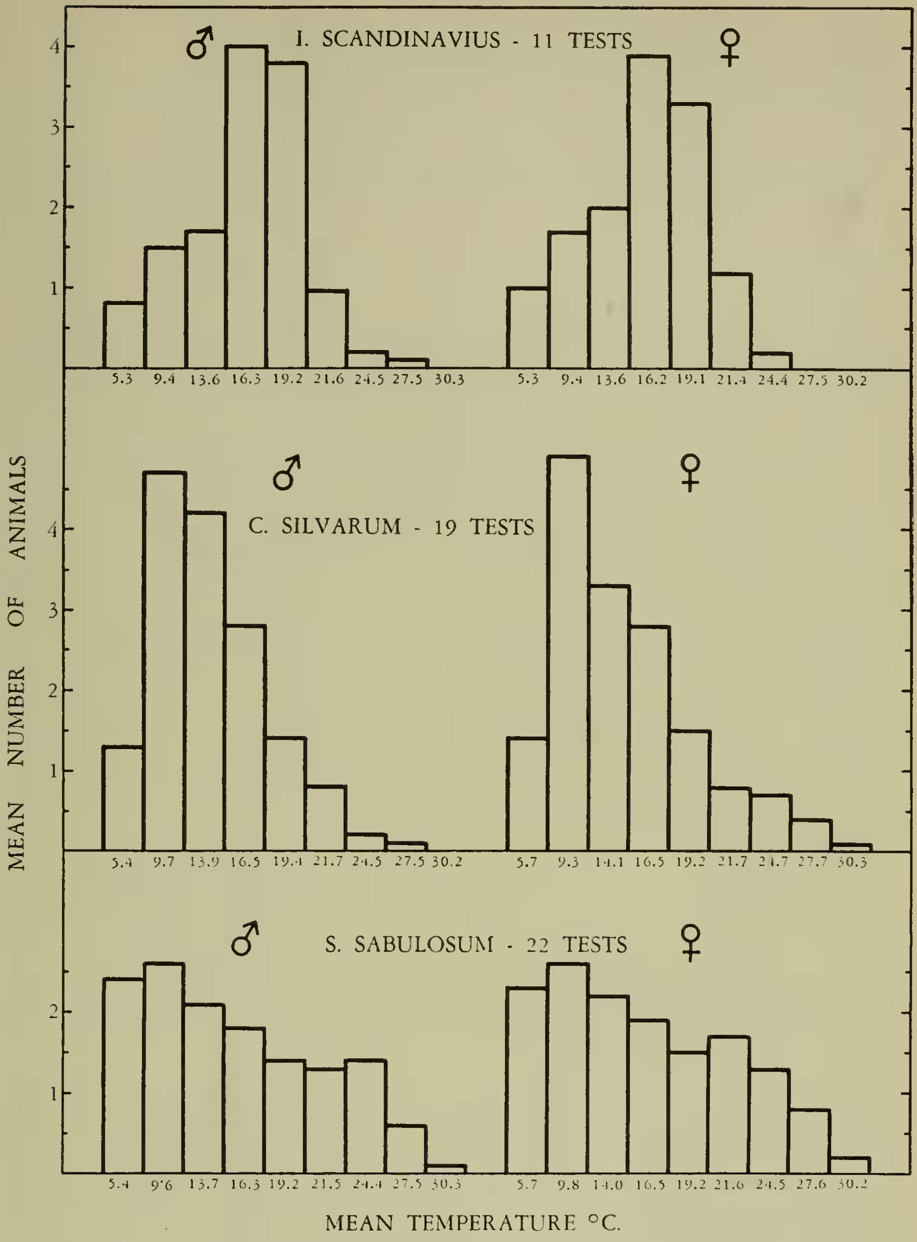


Fig. 21. Selection of temperature. Each figure represents the average results of all tests throughout the year. Sixteen animals were used in each test.

of seasonal variation in the temperature selections of *S. sabulosum* and *I. scandinavius* in the form of a narrowing of the selected temperature range and a consequent increase in the intensity of selection during the winter months. This results in a definite selection by *S. sabulosum* of the lowest temperatures in the range during January and December. This species showed little or no selection during the rest of the year. The intensities of selections by *I. scandinavius* and *S. sabulosum* males during the winter were higher than those of females of the same species, an occurrence which was noted also in humidity selection.

These relationships did not appear with *C. silvarum* which exhibited much the same distribution in the apparatus during the whole year.

DISCUSSION.

The interpretation of temperature preferences, determined by laboratory experiments, is a subject which deserves a good deal of consideration. The preferendum of individuals of a species may be variously affected by such factors as age, sex, the temperature to which they have been subjected before the experiment, whether they are well fed or not, and the state of their body water balance (DEAL, 1941). Besides the physiological state of the animals, experimental conditions are also influential. Humidity differences, which are concurrent with temperature changes in nature, must be eliminated if the effect of temperature alone is desired. Similarly, other environmental factors must be removed or standardized as far as possible. Results obtained must, consequently, be regarded with these facts in mind. It is particularly risky to accept the exact temperatures preferred by animals in laboratory experiments as those which they select under natural conditions. For instance, in the present experiments, *I. scandinavius*, tested in December, chose temperatures between 14° and 19° although temperatures in the field in December are considerably lower than this. A similar preference under natural conditions would lead the animals on an endless and fruitless search. In reality, they burrow into the ground in the fall and overwinter at temperatures likely substantially lower than those which they select in the laboratory.

Observations of temperature preference may be particularly enlightening in a comparison of the temperatures chosen by different species. In this respect, results become applicable to studies of the distributions of species in the field. Perhaps the most important considerations are those of range and intensity of preference since these factors indicate, to some extent, the reactivity of the animals to the temperatures which they encounter in their environments.

Of the three species of millipedes, *I. scandinavius* possesses the most intense reaction towards temperature differences which consequently have greater influence on its orientation than is the case with the other two species, although the difference between *I. scandinavius* and *C. silvarum* is not great in this respect. Temperatures between 5° and 30° are apparently of little concern to *S. sabulosum*, its only orientation, generally, being in a tendency to avoid the highest temperatures.

Eurytopic species may commonly choose higher temperatures than stenotopic species as found by VAN HEERDT (1950) and THÉODORIDES & VAN HEERDT (1952) with Coleoptera. In the present experiments, the widely distributed *I. scandinavius* chose higher temperatures than *C. silvarum* which has a more restricted distribution. *S. sabulosum*, however, exhibited a different manifestation of a eurytopic habit, namely, the absence of a definite temperature preference,

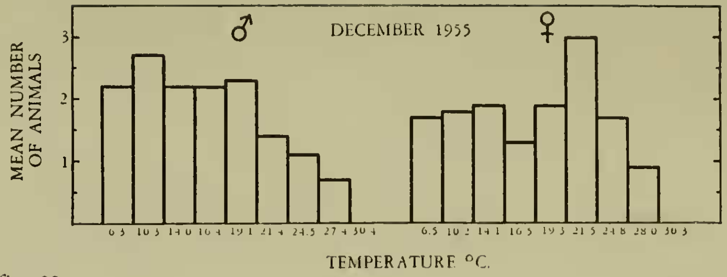


fig. 22

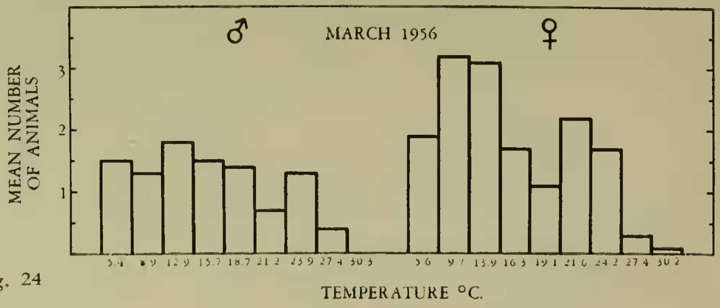


fig. 24

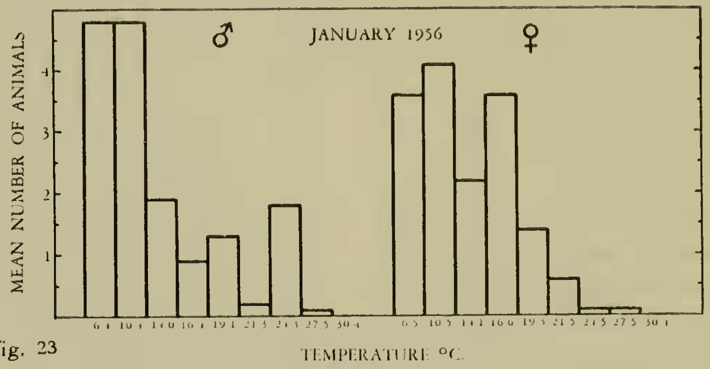


fig. 23

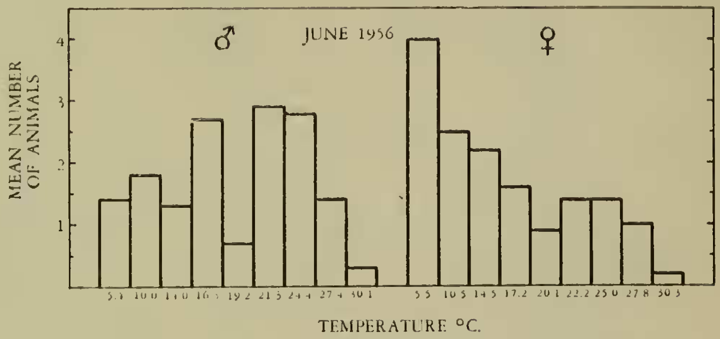


fig. 25

Figs. 22—25. Selection of temperature by *S. sabulosum* at different times of year. Each figure represents average results of three tests using 16 animals in each test.

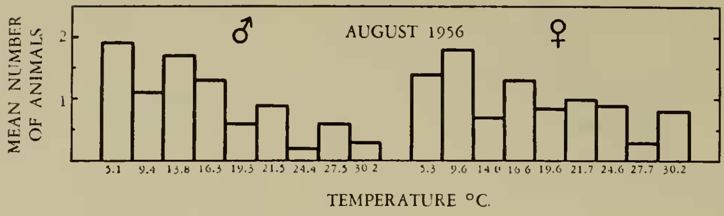


fig. 26

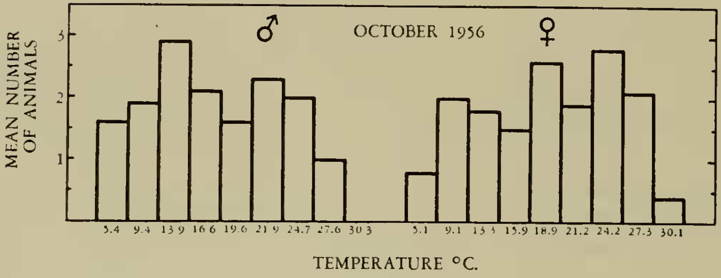


fig. 27

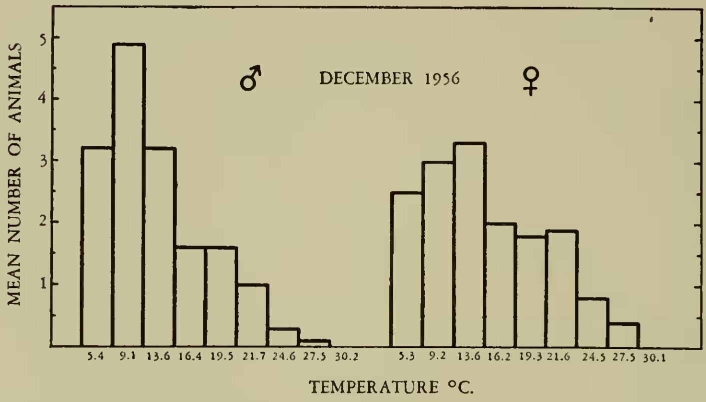


fig. 28

Figs. 26—28. Selection of temperature by *S. sabulosum* at different times of year. Each figure represents average results of three tests using 16 animals in each test.

within limits, indicating independence of environmental temperature within these same limits.

Temperature preference may have some influence in overwintering. During the winter the temperature of the soil probably becomes higher than that of the air and surface debris in which case the animals burrow into the ground, perhaps partly in response to the temperature gradient, towards their preferred temperature. In the spring, the temperatures of the air and surface litter become higher than that of the soil. The temperature gradient is thus reversed, leading the animals to emerge from the ground towards their preferred temperature. Certainly the initiation and termination of hibernation is more complex than this, as discussed in connection with humidity reactions, and it is likely that temperature preference has only minor influence in the overwintering process, particularly with *S. sabulosum* which exhibits only a very vague temperature preference.

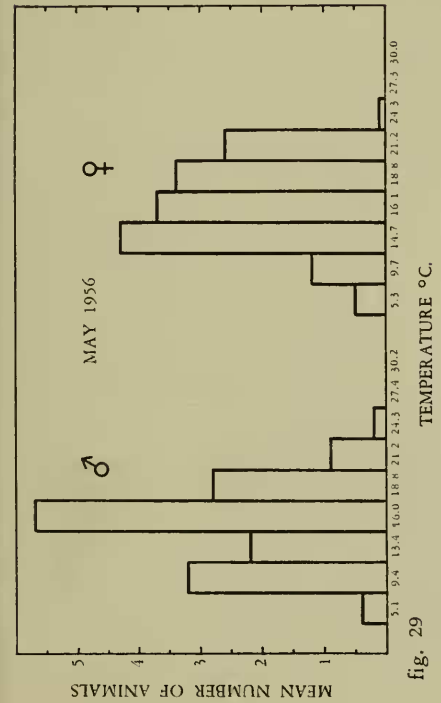


fig. 29

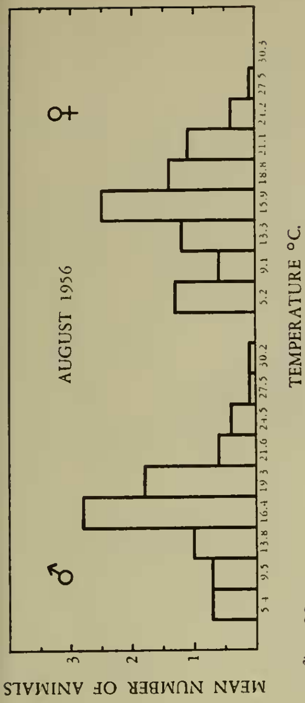


fig. 30

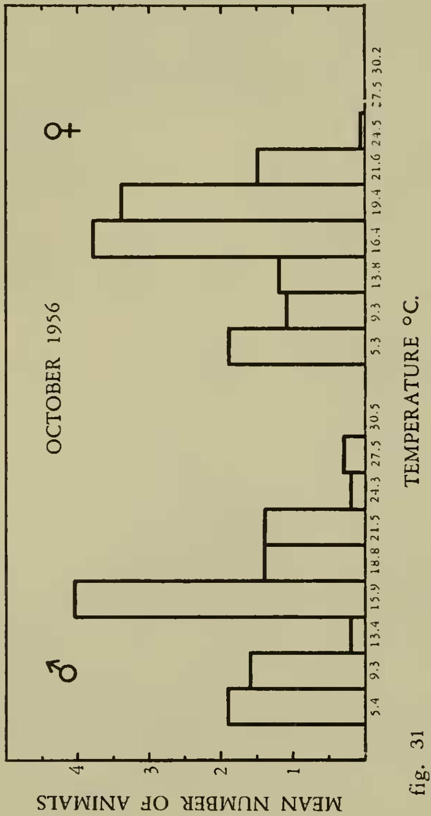


fig. 31

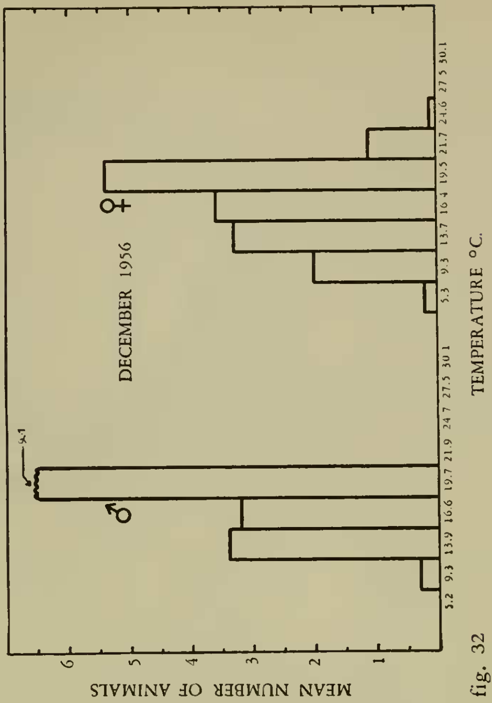


fig. 32

Figs. 29—32. Selection of temperature by *I. scandinavius* at different times of year. Each figure represents average results of three tests using 16 animals in each test.

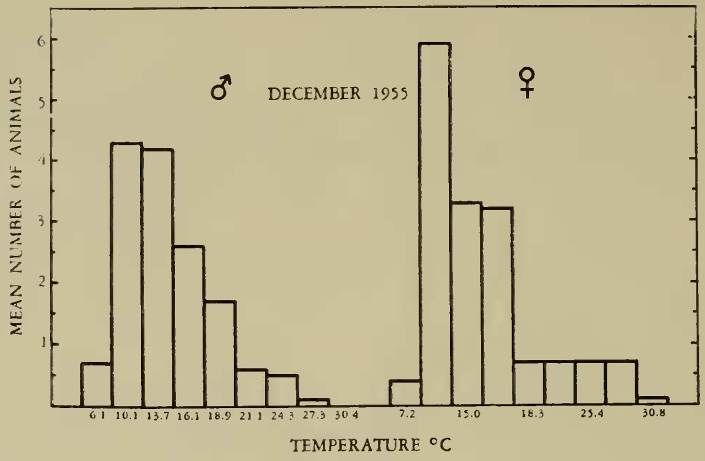


fig. 33

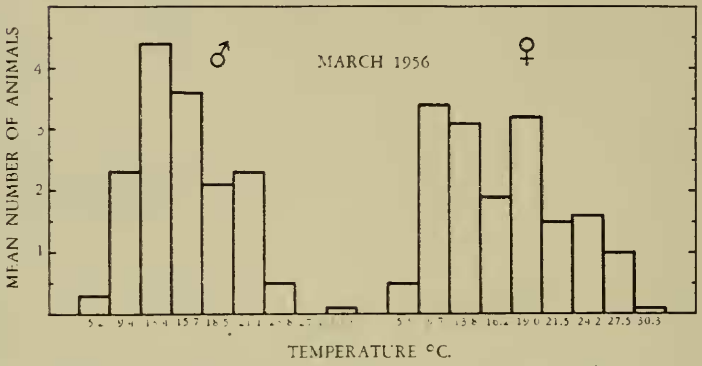


fig. 34

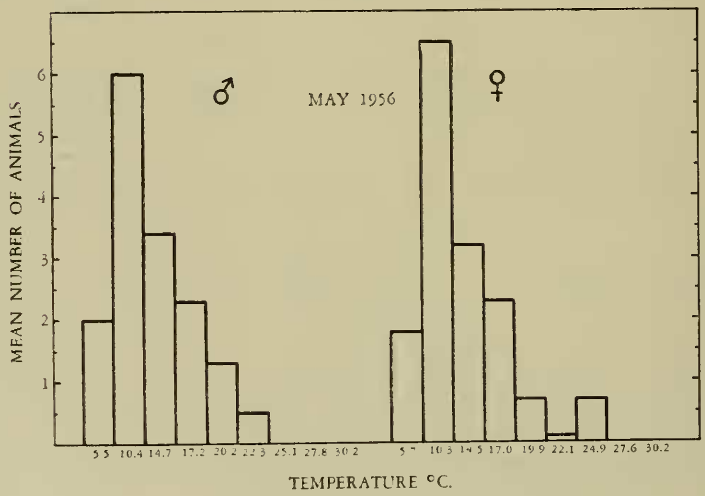


fig. 35

Figs. 33—35. Selection of temperature by *C. silvarum* at different times of year. Each figure represents average results of three tests using 16 animals in each test.

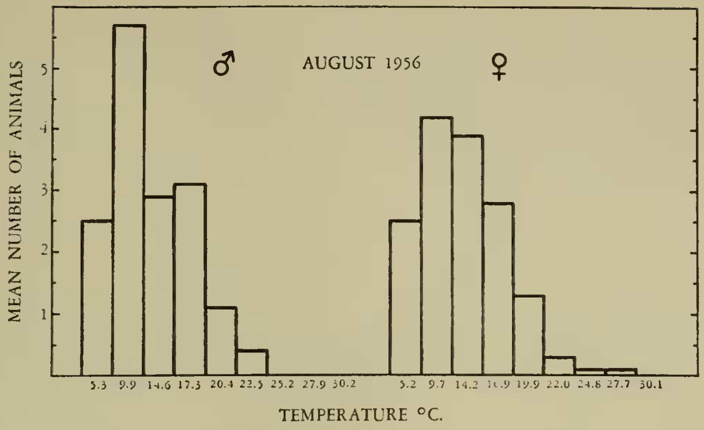


fig. 36

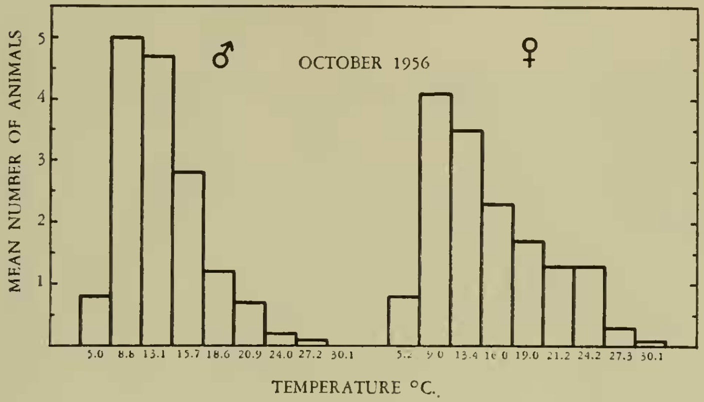


fig. 37

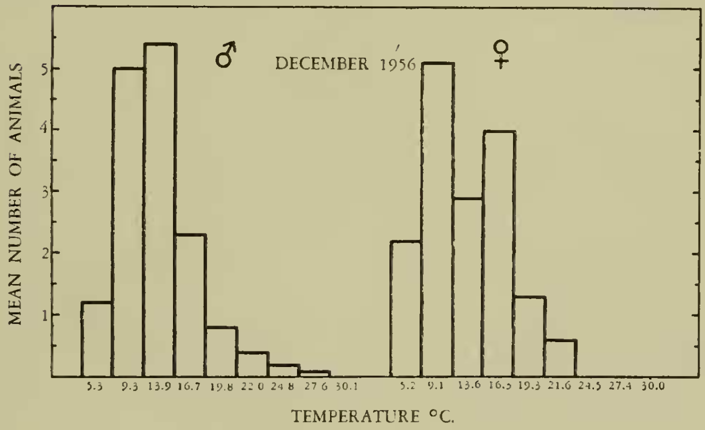


fig. 38

Figs. 36—38. Selection of temperature by *C. silvarum* at different times of year. Each figure represents average results of three tests using 16 animals in each test.

The temperature preferences of *S. sabulosum* and *I. scandinavius* become somewhat more pronounced during winter than at other times during the year while *C. silvarum* maintains an equally strong temperature preference throughout the whole year. The humidity preferences of *S. sabulosum* and *I. scandinavius* also show more regular seasonal changes than in the case of *C. silvarum*. The significance of such changes in environmental preference has been discussed in connection with humidity preference.

TEMPERATURE AND FEEDING ACTIVITY

The prolonged influence of temperature on the reactions and general metabolism of animals forms the second consideration of its importance as a habitat factor. Several facts resulting from an experiment to determine the influence of constant and alternating temperatures on the feeding activity of millipedes are worth mentioning.

METHOD.

Ninety males and 90 females of each of the three species were weighed and placed in groups of 10 in each of nine temperature conditions of a series thermostat. Constant temperatures of 5°, 10°, 15°, 20°, 25°, and 30°, with a saturation deficit of 1± mm of Hg were produced in six of the cells. The other three cells produced alternating temperatures which will not be considered here. The animals were maintained individually in small plastic boxes in the thermostat for 42 days. A small piece of potato was placed in each box as food. Every two days, a fresh piece of potato was added and the number of feces present was counted. Preliminary experiments indicated that the number of feces was a fair indication of the amount of food eaten. After 42 days, the animals which were still alive were reweighed and dried over P_2O_5. The dry weight was compared with the mean dry weight calculated, by the same method, for a group of animals of each species at the beginning of the experiment.

RESULTS.

Feeding Activity

Figs. 39—41 illustrate the relation between temperature and feces production with each species. Maximum feeding activity was shown by all three species at temperatures of 15° and 20°. Feeding activity increased with temperature up to 15°. Above 20° a difference existed between *I. scandinavius* and *C. silvarum*, on the one hand, and *S. sabulosum* on the other. The number of feces produced by *I. scandinavius* and *C. silvarum* dropped sharply to a low at 30°, but with *S. sabulosum* there was a relatively slight reduction after 20° and the feeding activity then remained on a level with that shown at about 10°. Also, at all temperatures except 5°, the mean number of feces produced by *S. sabulosum* was greater than that produced by either *I. scandinavius* or *C. silvarum*.

Body Weight

A second point of interest to be noted from this experiment is the relation between temperature and change in body weights of the animals which is presented

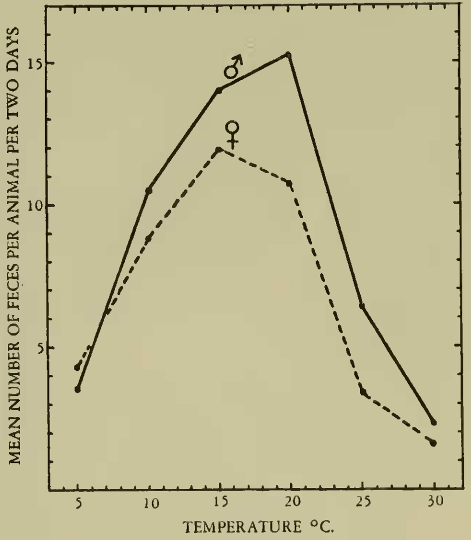


fig. 39

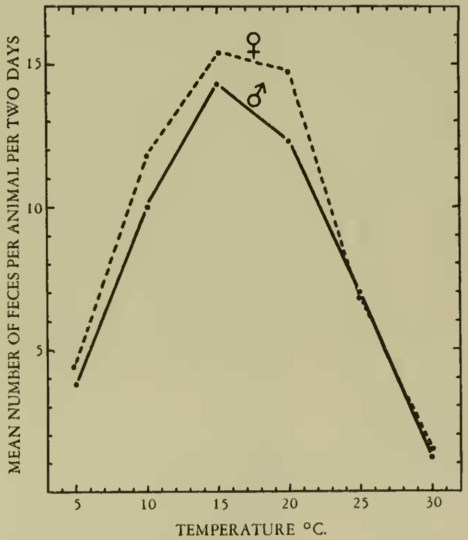


fig. 40

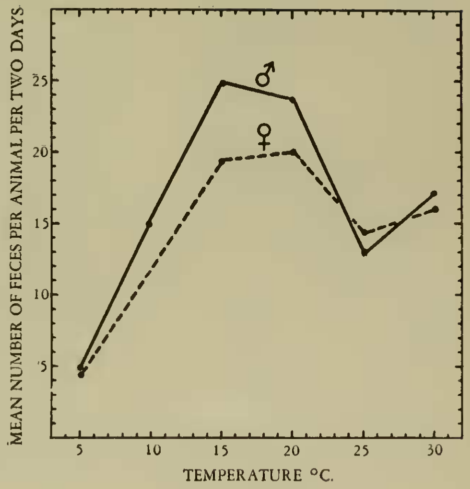


fig41

Figs. 39—41. Relation between temperature and feeding activity of (39) *I. scandinavius*, (40) *C. silvarum*, (41) *S. sabulosum* in Figs. 42, 43, and 44. *I. scandinavius* showed a loss in body weight at all temperatures except 10° and 20°, with males, and 10° and 15° with females; *S. sabulosum* gained weight at all temperatures; and *C. silvarum* increased in body weight at all temperatures below 25°, although the amount of gain decreased with rising temperature after 10° with the females and after 15° with the males. The drop in weight in the case of *S. sabulosum* at 20° was due to technical failure of the humidity apparatus in the cell during the last few days of the experiment with this species. This resulted in a high saturation deficit in the cell and a consequent loss of water by the animals. Disregarding this erroneous result, gains in body weight by individuals of this species did not decrease regularly with rising temperature as occurred with *C. silvarum.*

Some information concerning the nature of this weight change was derived by comparison of the dry weights of the experimental animals after the experiment with the mean percentage dry weight of a group of animals of each species at the beginning of the experiment. Percentage dry weight has been found to be a fairly constant and specific characteristic (Table I) so that this comparison is probably reliable within limits. The percentage dry weight of *C. silvarum* and *S. sabulosum* increased over the whole range of temperature with a consequent decrease in the percentage body water. With *I. scandinavius,* however, the percentage dry weight decreased, generally, with a resultant increase in percentage body water.

Although a loss or gain in body weight may be due to the simultaneous decrease or increase of both body water and dry material, these results suggest that the gains in body weights of *C. silvarum* and *S. sabulosum* during the experiment were likely due to a proportionally larger increase in dry material than in water. The general weight loss of *I. scandinavius*, similarly, was comprised probably of a relatively greater loss of dry material than of water. Because of the slow rates of growth of these three species plus the fact that most of the individuals were full grown, changes in the amount of dry material in their bodies during this experiment were probably due to increase or decrease in the fat reserves of the animals.

Mortality

The number of individuals of each species which died at each temperature during the experiment is given in Table III. *I. scandinavius* displayed generally higher mortality at all temperatures than the other two species and the instance

TABLE III. Mortality of millipedes during 42 days at different temperatures and a saturation deficit of 1 ± mm Hg. Animals were fed potato.

Temperature ° C.	*C. silvarum*		*I. scandinavius*		*S. sabulosum*	
	♂	♀	♂	♀	♂	♀
5	—	—	—	2	—	—
10	1	—	—	1	1	—
15	1	—	2	2	—	—
20	1	1	5	4	—	—
25	—	1	2	6	—	—
30	3	—	8	7	—	1

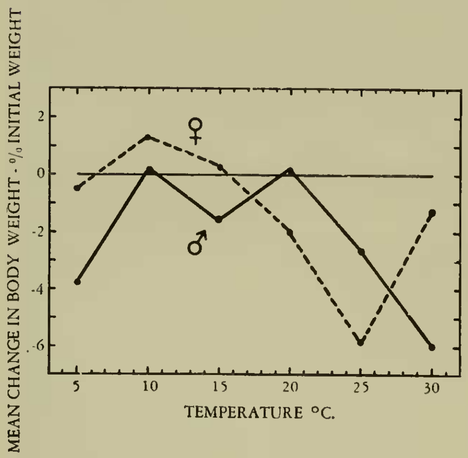


fig. 42

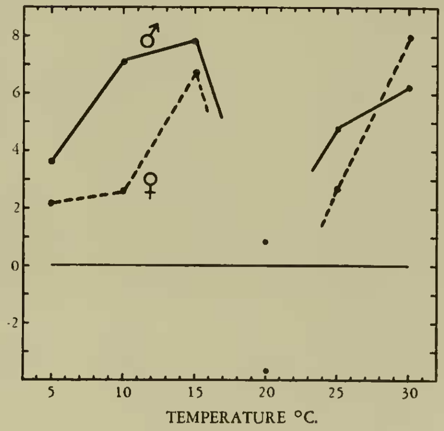


fig. 43

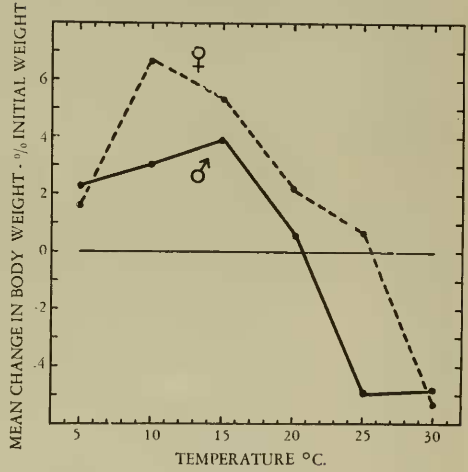


fig. 44

Figs. 42—44. Relation between temperature and change in body weight during 42 days; 42, *I. scandinavius,* 43, *S. sabulosum,* 44, *C. silvarum* of mortality increased with temperature. Fewer deaths occurred during the experiment with *C. silvarum* and fatalities were not as obviously a function of the experimental temperature. Mortality of *S. sabulosum* during the experiment was negligible.

DISCUSSION.

The general impression created by the results is similar to that derived from temperature preference experiments, namely, that *C. silvarum* and *I. scandinavius* are affected more intensely by temperature than *S. sabulosum.* The temperature at which a maximum amount of food was eaten was the same with all three species but this activity was only slightly altered by different temperatures in the case of *S. sabulosum* while the higher temperatures became increasingly depressive for *C. silvarum* and *I. scandinavius.*

Also, *S. sabulosum* increased in weight with much the same magnitude at all temperatures. *C. silvarum* increased in weight during the experiment at all temperatures below 25°. *I. scandinavius* males lost weight at all temperatures except 10° and 20° where their weights remained about the same; females lost weight at all temperatures except 10° and 15°. The loss of weight by individuals of this species over almost the whole range of temperature probably reflects a general lack of resistance in *I. scandinavius* towards artificial conditions.

Mortality during the experiment presented much the same picture of the influence of temperature on the three species.

LETHAL TEMPERATURE

The previous experiment was concerned with the prolonged influence of various temperatures on certain processes. In the following investigation, the upper lethal temperatures of the three species of millipedes were determined.

METHOD.

Determinations of lethal temperature are complicated by the influence on the experimental animals of a loss of body water which, if prolonged, also becomes a lethal factor. To eliminate this influence it is necessary to maintain 100% relative humidity around the experimental animal. Secondly, it is necessary to standardize the time of exposure of animals to each temperature during the tests.

Following a method used by HOLM (1952), animals were tested individually in a test tube of about 3 cm diameter. The tube was stoppered with cotton-wool through which a thermometer was projected so that the bulb hung about one cm from the bottom of the tube. The cotton-wool was moistened and the inside of the tube down to two or three cm from the bottom was lined with damp filter paper. The upright tube was submerged for about two-thirds of its length in a beaker of water which was slowly warmed, at a rate of one degree per minute, by a small gas flame. Each test was begun with the water in the beaker at 20°. During tests, the water was kept in motion by an electric stirring apparatus. Experimental animals were observed through a magnifying lens. The temperature at which the last movement of the animal was observed was taken as its lethal temperature. Although sometimes difficult to determine exactly because of the curled up posture assumed by the animals shortly before death, this point was found to be a good criterion of death. Rarely did an animal recover when returned to room temperature.

Results and Discussion.

Twelve *C. silvarum* and 15 individuals of each of the other two species were tested. No difference was found between the lethal temperatures of the sexes in any of the three species. The means and standard errors of the lethal temperatures of each species calculated from figures for both males and females are as follows: *C. silvarum* 43.6 ± 0.45°, *S. sabulosum* 41.7 ± 0.17°, and *I. scandinavius* 40.7 ± 0.13°. Differences between these means are not large but are statistically significant. There was little variation between individual observations.

Assuming the results to be indicative of resistance to high temperature, *C. silvarum* is apparently superior to the other two species in this respect. If this is so, it may be concluded from consideration of the habitats of the three species that other factors are responsible for the limited distribution of *C. silvarum* in the dunes.

Again in this experiment, *I. scandinavius* proved to be most susceptible to the effects of unfavourable environmental conditions. This phenomenon, which has appeared repeatedly throughout all the experiments, is also ostensibly in opposition to the wide distribution of the species and will be discussed in a later section.

Although lethal temperatures have been widely determined for insects (reviewed by Uvarov, 1931), the importance of this characteristic as a consideration in the distribution of animals is questionable, since, as usually defined, it does not consider the sustained influence of temperature on an animal. In most cases in nature an animal's avoiding reactions would prevent it from entering an area where the temperature was high enough to be rapidly lethal. It is, rather, the physiological effect of heat over a period of time, as considered in the previous experiment, and on different stages of the life cycle, which is ecologically important. The effect on animals of prolonged exposure to extremes of temperature is a function of their heat or cold hardiness, as distinct from heat or cold death. Quite different factors may be involved, for instance, in relatively sudden death by exposure to very high temperatures (Heilbrunn et al., 1946) and a more gradual collapse due to inefficient action of physiological systems at unsuitably high temperature. Also, heat or cold hardiness may depend on certain behavioural or physiological adaptive processes which are usually not operative in determinations of lethal temperatures. These include migration, burrowing, nocturnal habits, evaporation of water from the surface of the body, hibernation of one form or another, and acclimatization (Allee et al., 1949).

As a limiting factor in the distributions of the three species, temperature probably has less influence, generally, than humidity. Laboratory experiments have shown that reactions towards temperature are usually less pronounced than reactions towards humidity and are correlated less closely with seasonal variations in behaviour. This relationship may logically be expected in a temperate, coastal climate where temperature variations during the year, above or below the normal activity range, are seldom prolonged. But, as mentioned previously, the realization of and reaction to humidity changes in the environments of these millipedes is constantly necessary since they tend to lose water at all humidites below saturation.

SUMMARY

I. scandinavius and *C. silvarum* showed definite preference for certain temperatures between 5° and 30° but *S. sabulosum* tended only to avoid the highest temperatures in the range. *I. scandinavius* chose the highest temperatures of the three species and also preferred the narrowest range of temperature.

The preference of males was similar to that of females.

No seasonal variation was recorded in the actual temperatures selected but preference became slightly more pronounced during winter.

Maximum feeding activity was exhibited by all three species at the same temperature but *C. silvarum* and *I. scandinavius* were more strongly affected by variations in temperature than *S. sabulosum.*

The highest lethal temperature was found for *C. silvarum* and the lowest for *I. scandinavius.* It may be concluded from this that other factors are reponsible for the limited distribution of *C. silvarum* in relation to the other two species.

Surveying results of experiments on both temperature and humidity reveals a general similarity between the reactions of each of the three species towards these two factors. *S. sabulosum* proved to be least dependent on either temperature or humidity while *I. scandinavius* showed the strongest reactions towards the two factors and *C. silvarum* was usually intermediate in this respect. This resemblance between reactions towards both temperature and humidity can reasonably be expected since the two factors are actually inextricably combined under natural conditions. Relative humidity is usually inversely dependent on temperature. Changes in temperature are probably almost always indicative of corresponding changes in humidity of the air except in habitats where there is water in excess of the amount required to saturate the air with water vapour at all temperatures in the normally occurring range. Animals whose distribution is strongly affected by humidity can be expected, thus, to occur in areas where normally experienced fluctuations in temperature do not produce humidity conditions which are unfavourable. Also, animals with restricted temperature requirements will be adapted, either through behaviour or physiology, to humidities occurring within that temperature range in which they normally are found.

FOOD

This subject seems to have received more attention, though superficial, than other aspects of the ecology of diplopods. As early as 1884 LATZEL had summarized the food habits of millipedes. VERHOEFF (1914) referred to millipedes as poecilophagus (omnivorous) which may be true in some instances but does not appear to be a general characteristic of the group. Most of the publications on the food of millipedes have been concerned with *Blaniulus guttulatus* Gerv. and *Diploiulus londonensis* var. *coeruleocinctus* (Wood), two species which have attracted the most attention because of their occurrence among economically important crops. Papers by BRADE-BIRKS (1930), CLOUDSLEY-THOMPSON (1950b), and KINKEL (1955) gave the food of *B. guttulatus* as consisting of dead and rotting plant material. But at times this species also appears to attack living plants and is, therefore, generally considered an agricultural pest although damage done by the species is apparently never extensive. Attacks on potatoes, sugar beets, oats, wheat, cucumber, carrots, strawberries, and young shoots of beans and peas have been reported. According to some authors, *B. guttulatus* begins to feed on crop plants only after rotting has been initiated by other parasites such as slugs, parasitic worms, etc. Because most cases of crop attack by millipedes occur during periods of drought, it has been suggested that the primary cause of such outbreaks is the search for new, suitably humid environments when the litter has become too dry. Besides vegetation, this species has been reported also to feed on dead slugs and worms and *Collembola* are often found in the gut. KINKEL (l.c.) found that individuals which had been kept for a time in the laboratory on a protein-free diet began eating the legs off their fellows as well as the remains of damaged legs on their own bodies. He also observed that they readily ate molds.

D. londonensis coeruleocinctus, a species which has spread from Europe to many parts of North America, also lives predominantly on rotting vegetation but occasionally eats a number of living plants according to BRADE-BIRKS (1930). He found that they ate potato and molds in the laboratory. Crop damage incurred by this species may be considerable. MACLEOD (1934) reported 15% to 97% injury to potato fields in New York.

LYFORD (1943) stressed the importance of millipedes in the decomposition of fallen leaves and in the forming of the so-called "mull" layer of the forest floor although small insects, worms, and bacteria are responsible for probably 95% of this breakdown process. He investigated the possibility that a preference for certain kinds of leaves as food exists among millipedes and claimed to have found a positive relation between the extent to which a certain kind of leaves were eaten and the Ca content of the leaves. His figures are not convincing, however. Previously CHANDLER (1939) had reported a similar preference of diplopods for leaves with high Ca content.

Less is known about the feeding habits of the diplopod species concerned in the present work. All the pertinent literature can be briefly summarized. According to VOM RATH (1891), molds are the chief source of food for *S. sabulosum*. SORAUER (1913) reported that *S. sabulosum* climb in shrubs where they eat leaf parenchyma. This assertion, if referring to fresh, green, leaves, seems unlikely in view of the succeeding experiments with this species. VERHOEFF (1914) saw *S. sabulosum* climbing in *Ranunculus* during the day and claimed that they ate pollen from the flowers. This observation should be regarded with reserve too since it is known that millipedes occasionally hide in flowers during the day without necessarily feeding on them. VERHOEFF also regarded living leaves as an unlikely source of food for *S. sabulosum* and BRADE-BIRKS (1930) stated that, during laboratory experiments, the animals ate filter paper rather than living strawberry leaves even though the paper was not digested. In the same publication, BRADE-BIRKS mentioned that *C. silvarum* occur under the bark of dead trees where they eat mainly wood. During experiments on the nutrition of this species, VAN DER DRIFT (1950) reported that the animals grew faster when fed the F_1 litter layer than when maintained on the F_0 layer. Recently fallen leaves were not eaten.

Apparently nothing has been published on the food habits of *I. scandinavius.*

From the existing literature may be concluded that the main food of millipedes is dead vegetation supplemented occasionally with soft, living plant material. Animal remains are apparently not entirely excluded. Preference for one particular substance as food has never been reported.

The purpose of the following study was to discern the importance of food as an influence in the distributions of the three species of millipedes and as a factor in their ecology. The types of food eaten were determined by analysing the contents of feces produced by the animals and attempts were made to discover whether preferences exist for certain substances as food and whether light and darkness and variety of diet influence the amount and choice of substances eaten. A certain amount of information was also obtained on the digestive process in millipedes.

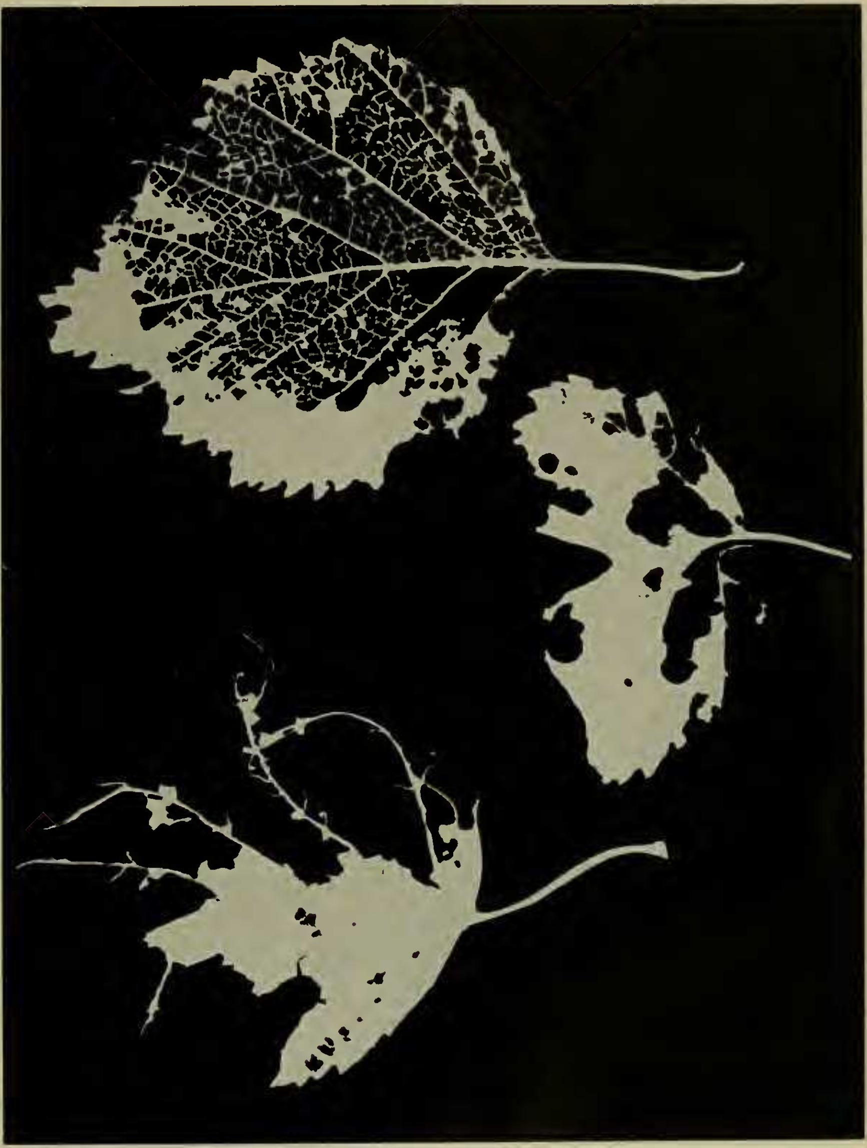
Substances Accepted as Food

To discover what is eaten by the diplopods under natural conditions, it was necessary first to determine the appearance of known substances after passing through the digestive tracts of the animals. A number of plant materials considered to be likely foods were collected from an area of birch woods where experimental animals were also obtained and were then fed to millipedes in the laboratory. The substances used were :

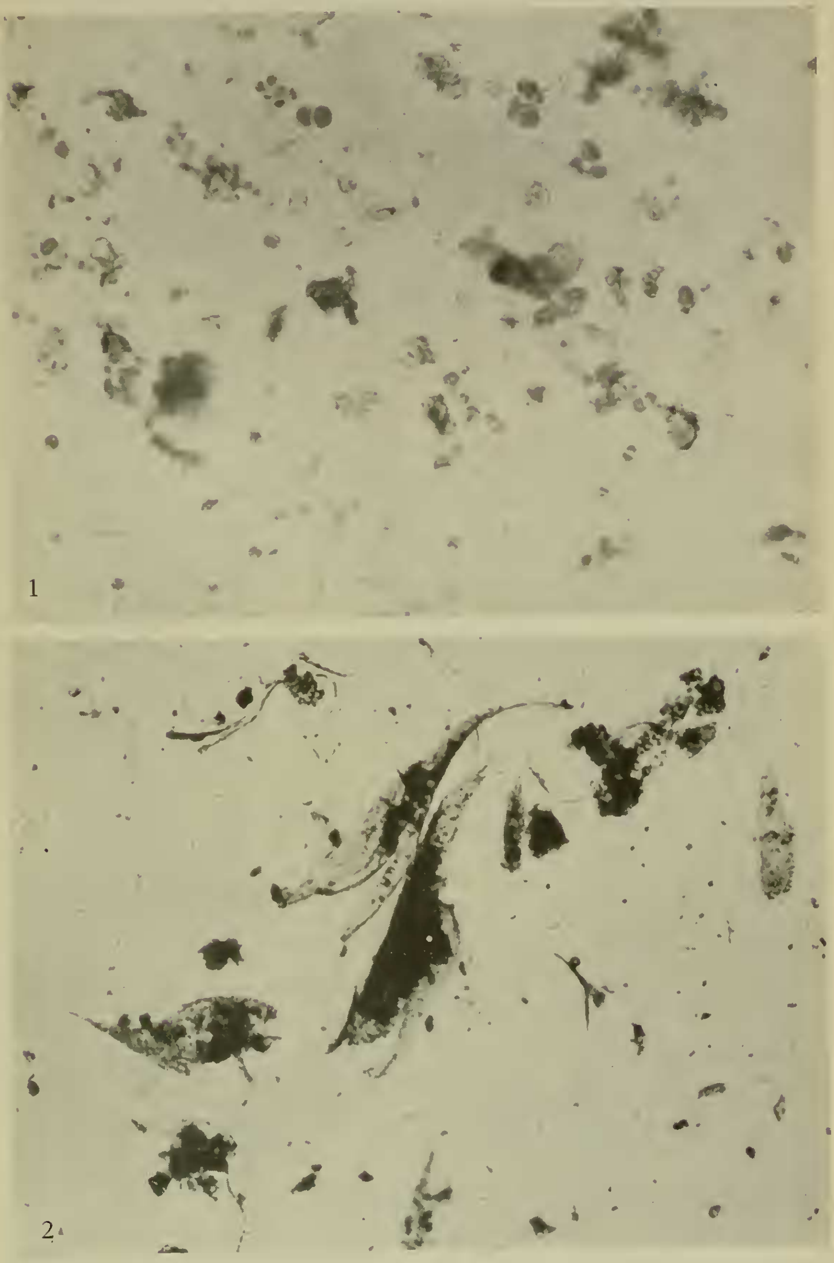
Betula verrucosa Ehrh.
Rotting leaves — *Populus tremula* L.
Crataegus monogyna Jacq.
Living leaves — *B. verrucosa*
P. tremula
Dead wood — *B. verrucosa*
Rotting wood — *B. verrucosa*
Lichens — *Parmelia sulcata* Tayl.
Parmelia physodes (L.)
Moss — *Brachytecium rutabulum* (Hedw.)
Pleurococcus
Dead grass
Aspergillus sp.
Wood fungus

These substances were, of course, not the only potential foods in the area but were chosen as the most likely because of their relative abundance. The feces produced by individuals of the three species maintained on these substances were collected and examined microscopically.

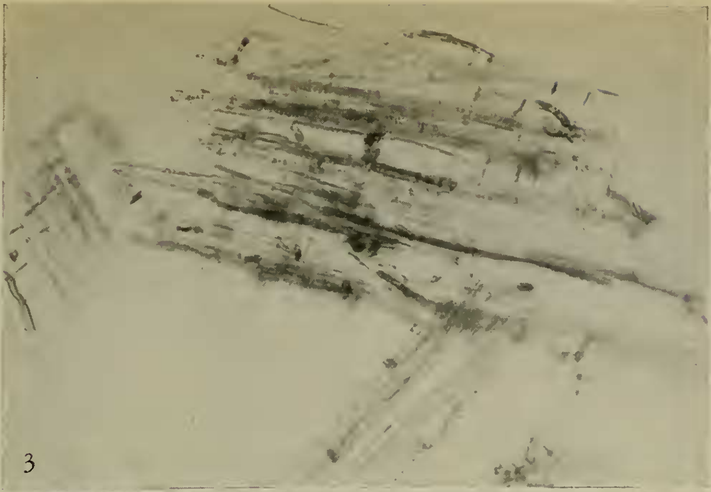
These preliminary experiments provided primarily an impression of the substances accepted by the three species as food and of the relative degree to which these materials are eaten. With the exception of living leaves, dead wood and the one species of fungus, all the substances were accepted, to some extent, as food. The animals ate moist filter paper rather than living *B. verrucosa* and *P. tremula* leaves. Rotting leaves of these two species, the mold *Aspergillus,* and pleuro-



Pattern of leaf consumption characteristic of, from top to bottom, *C. silvarum*, *I. scandinavius*, *S. sabulosum*



Photomicrographs of contents of feces. Fig. 1, cells of Pleurococcus; Fig. 2, moss fragments



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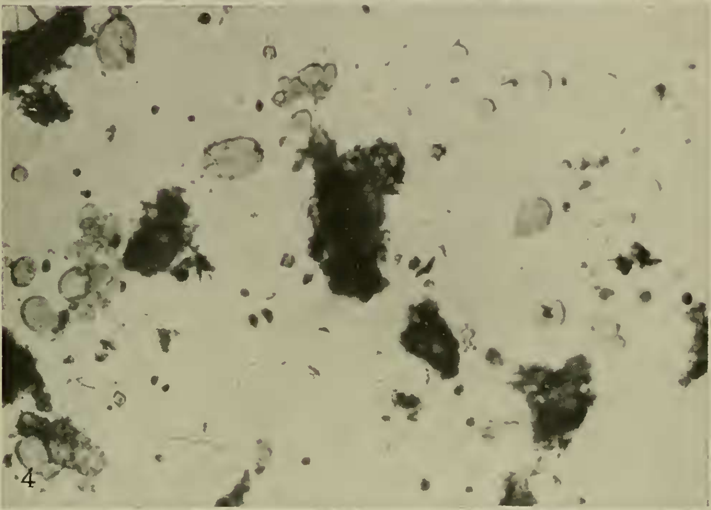

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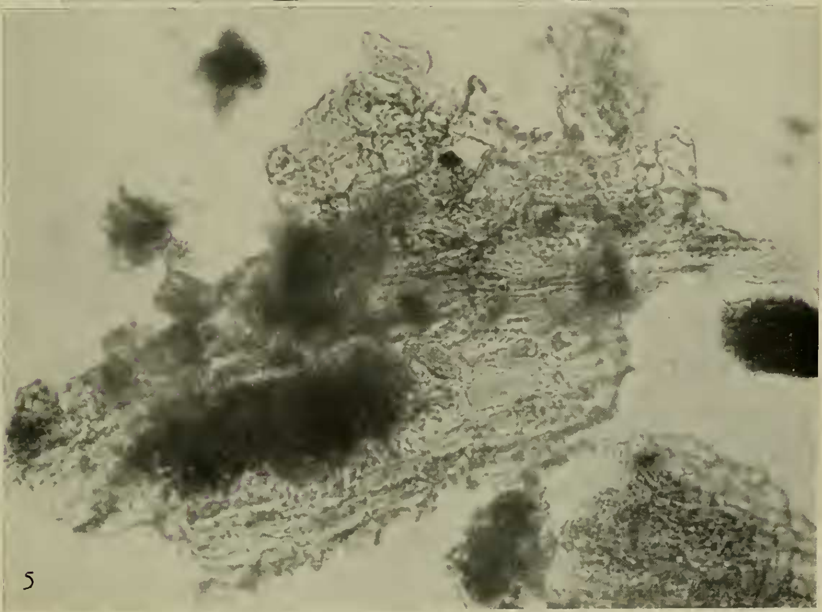

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Photomicrographs of contents of feces. Fig. 3, wood fragments with xylem vessels clearly visible; Fig. 4, large globulate starch-storing cells; Fig. 5, leaf fragment showing vein and adjacent parenchyma

coccus were eaten abundantly by all three species. *I. scandinavius* ate rotting wood sparsely, but the other two species consumed it readily. *S. sabulosum* and I. *scandinavius* ate *Parmelia* especially well. Although little grass was eaten during the tests, this substance was sometimes found in feces collected from *S. sabulosum* and *I. scandinavius* in the field and is thus an accepted food under natural conditions. Fungus was not eaten by the millipedes in the laboratory although individuals of the species *B. guttulatus* are often found in such saprophytic growths. However, this may be a case of microhabitat selection rather than food preference.

PALATABILITY.

In several instances during this study, a certain piece of rotting wood or rotting leaf was not accepted as food although there was no obvious difference between it and other similar substances which were eaten. The reason for this phenomenon, which was observed also by LYFORD (1943), is obscure.

Dead wood was apparently impalatable or inedible. In experiments in which a piece of dead wood and a piece of rotting wood of the same species were offered side by side, only the rotting wood was eaten even if it was dry and the dead wood was moist. In such cases the hardness of the wood may have been the factor determining its acceptability as food. The xylem vessels in rotting wood, although intact, can be pulled apart easily. Wood probably becomes edible for millipedes only after it has been softened by bacteria and molds.

A difference in the palatability of different tissues of a rotting leaf was also strikingly apparent from the way in which individuals of each species consumed a leaf according to a characteristic pattern (Plate 10). *C. silvarum* ate only the soft leaf parenchyma, *S. sabulosum* ate the parenchyma plus the small, anastomosing nerves, but not the larger, central and side nerves, and *I. scandinavius* ate the whole leaf. This consumption pattern was exhibited only when sufficient food was available. Starved animals of all three species ate the whole leaf. It seems unlikely that the hardness of different leaf tissues could be of influence in this feeding pattern since *C. silvarum*, which are characteristic wood eaters, ate only the softest part of the leaf while *I. scandinavius*, which generally eat very little wood, consumed the whole leaf.

The factors which determine the palatability or edibility of a substance for millipedes are not known. In general, a certain softness must be necessary because of the physical limitations of the chewing mechanism. This factor may be of particular influence in the case of wood. The impalatability of living leaves, however, could hardly be ascribed to their hardness or toughness since individuals of all three species eat a certain amount of wood which is harder than leaf tissue. The accessibility of living leaves is also not a factor in their use by millipedes as food. Although the animals are confined in their movements during the day, they move about relatively freely at night when they regularly climb small trees and bushes. The moisture content of a substance is apparently sometimes important although, again, this is not the complete answer. Moist, fresh, leaves are rejected in favour of dry, rotten ones. Whatever the factors are which decide the palatability of a substance for millipedes, they must be rather general because of the wide variety of materials which are eaten.

DIGESTION.

Examination of the feces revealed that relatively large pieces of food pass the mandibles. The size of the feces components varies with the size of the animal; leaf fragments as large as 0.5 mm in diameter can sometimes be found. Digestion appeared to be incomplete and to have little influence on the cells of the substances consumed which were recovered practically unaltered in the feces. Algal cells (Plate 11, Fig. 1) in the feces were intact and still possessed their bright green colour. Similarly, moss fragments (Plate 11, Fig. 2) were still green and apparently unchanged. There was also little observable change in wood and leaf fragments (Plate 12, Fig. 3 and 5) although a number of the cells sometimes appeared to be plasmolysed. It is possible, however, that this plasmolysis was due to drying of the feces before examination.

The possibility that cellulose and lignin were digested was tested by staining normal and defecated leaf and wood cells with chlorozinciodine (cellulose) and phloroglucine in hydrochloric acid (lignin). No difference was observed between cells from the feces and uneaten cells. Evidently cellulose and lignin were not digested to any measurable extent. How these animals obtain their proteins is not clear. Supposedly, certain proteases must exist in their digestive tracts but, as far as is known, only fat and starch digesting enzymes are present in the gut of millipedes (RANDOW, 1924). Even so, digestion of starch was not general in the present experiments, large starch-storing cells were sometimes found in the feces (Plate 12, Fig. 4). Thus cellulose, lignin, and starch are apparently not absorbed to any extent during digestion and it seems conclusive from the condition of substances after passing through the digestive tract, that a very small proportion of the food is digested. This fact, if characteristic among millipedes, makes them efficient instruments in the mechanical breakdown process of the litter layers of the soil since a large percentage of the substances eaten by them is returned unchanged to the soil.

EXAMINATION OF FECES FROM MILLIPEDES IN THE FIELD.

Individuals of the three species were collected both during the day and at night, the substrate on which they were found was noted, and the contents of the feces produced by them during the next 24 hours were determined. The duration of the digestive process is variable depending on, among other things, the feeding activity of an individual. In cases of lack of food, substances appear to be retained in the gut for some time and pellets may still be produced after 35 hours fasting. During active feeding, however, the passage is completed in about two hours. It is thus likely that the feces collected in the field were produced from substances eaten by the animals during the last few hours before collection and can be designated with some degree of certainty as substances eaten during the day or at night.

Results are presented in Tables IV—VI in which the numbers are percentages of the total number of feces examined in which the particular substance was found. Since the composition of a pellet was sometimes heterogeneous, the percentages for any group of feces may sum more than 100.

Evidence found in the feces indicated that all the substances which had been eaten during the preliminary experiments in the laboratory are also eaten regularly

TABLE IV. Analysis of feces from *C. silvarum.*

Where collected	Wood	Leaves	Parmelia	Mold	Algae	Plant hairs	Moss	Animal fragments
Animals collected during the day								
In dead wood 302 feces . . .	100 %			2 %				
In dead wood on the ground 63 feces .	95 %	10 %	2 %	9%				
Animals collected at night								
On tree trunks 148 feces . . .	7%	8%	12 %	10 %	30%	14 %	47 %	2 %

by the animals in nature. Generally, a wide variety of substances is eaten by the three species. More green plant material and rotting leaves are consumed by *S. sabulosum* and *I. scandinavius* than by *C. silvarum.* The results pertain only to animals collected in one type of habitat, a birch and aspen wood, and it is possible that further examination of feces from animals in other habitats would reveal more substances which are regularly eaten.

TABLE V. Analysis of feces from *S. sabulosum*

Where collected	Wood	Leaves	Parmelia	Mold	Algae	Grass
Animals collected during the day						
In dead wood on the ground 48 feces	23 %	48 %	36 %		53 %	2 %
Animals collected at night						
On the ground and on tree trunks 156 feces . . .	19 %	7 %	94 %	1 %	4 %	

There also appeared to be some difference between the amount and variety of substances eaten during the day and at night. *C. silvarum* collected during the day produced feces composed almost entirely of wood. At night a striking increase was observed in the variety of substances found in the feces which were

TABLE VI. Analysis of feces from *I. scandinavius*

Where collected	Wood	Leaves	Parmelia	Moss	Animal fragments	Grass
Animals collected during the day						
In dead wood on the ground 198 feces	4 %	87 %	23 %	6 %	1 %	1 %
Animals collected at night						
On the ground and tree trunks 23 feces	31 %	78 %			9 %	

composed principally of moss and algae with very little wood. Diurnal differences in the composition of feces from *S. sabulosum* and *I. scandinavius* were not so obvious although a large proportion of the food of *S. sabulosum*, collected from tree trunks at night, consisted of *Parmelia* sp. More wood and leaf fragments were found in feces collected from animals during the day. These results are not presented as proof that a diurnal change in diet is necessarily general in the three species. Collections at night were composed entirely of animals found on tree trunks. These individuals represent certainly only a small proportion of the total population. Undoubtedly, many animals spend the night in dead wood or on the ground where their diet may be different from that of animals climbing in trees. But since nocturnal activity is characteristic of millipedes, they may in fact eat a wider variety of substances at night than during the day even though the proportions may be different than those shown in Tables IV—VI.

Food Preference

Method.

Should food be an important factor in the distributions of the three species, one would expect them to show preference for certain substances as food. To determine whether or not this is true, experiments were done in which millipedes were allowed to choose between a number of substances which had been found to be regularly eaten. These substances were attached to circular plaster of paris bases and equally spaced around the circumference of a large petri dish. Wet filter paper on the inside surface of the lid provided a high relative humidity within the dish. Each food was moistened with water before an experiment to minimize moisture differences. Foods were renewed regularly. A certain number of animals was released into the center of the dish, the lid replaced, and the dish enclosed in a box to shut out light. The number of animals on each food was noted every half hour for a number of hours. Between 100 and 200 observations in 10 to 20 tests were made with each sex of *C. silvarum* and *S. sabulosum*. Because of a scarcity of *I. scandinavius*, fewer tests were done with this species. Animals were kept without food between the tests.

The reliability of this method as an indication of the foods eaten was ascertained by comparing the results with those obtained from a series of experiments in which individuals were allowed access to foods for a number of hours after which the foods were removed from the dish and the contents of the feces produced were determined. Results of these tests were very similar to those obtained by the first method which was accepted and used throughout all experiments. With this method, it is possible that unavoidable differences between the surface textures of foods may influence results and care must be taken to discern between food preference and a possible preference for a certain substrate. Fastening the substances to plaster of paris alleviates this difficulty somewhat by preventing individuals from crawling under the foods.

The ventral, rhizoid possessing surfaces, and the dorsal surfaces of the lichens, *P. sulcata* and *P. physodes* were presented separately in the tests and are designated in the results as "d" (dorsal surface) and "v" (ventral surface).

RESULTS AND DISCUSSION.

Data are summarized in Tables VII—IX. The figures are means with their standard deviations representing the average number of animals observed eating each food. The mean values were compared statistically and the substances are divided into primary ⋮ and secondary [ ] selection categories. Numbers in the same category are not significantly different. In Table VII, for instance, the mean numbers of *C. silvarum* males observed eating wood and *P. sulcata* v. are statistically equal and are thus designated as a primary or first order selection. But the mean number representing the selection of *P. sulcata* v. is not significantly different from the means for the second and third *Parmelia* choices. Therefore the first three sources of lichen are grouped into a secondary or second order selection. This analysis shows, then, that wood and *P. sulcata* v. were eaten with equal frequency as were *P. sulcata* v., *P. physodes* d., *P. physodes* v., and leaf, *P. sulcata* d., and moss. Wood and the first three *Parmelia* choices were preferred above leaf, *P. sulcata* d. and moss.

Two points are immediately evident from the results. First, that no dominant preference for one particular food was shown by any of the three species. Any preference was a question of groups of substances. This fact is of prime importance since it indicates that food preference has little influence as a limiting factor in the distributions of the species. The second general similarity in the results was the enormous variation in the observations, a fact which inhibits a valid comparison of the results with different foods and also indicates the lack of an exaggerated preference for any one substance.

TABLE VII. Food preference of *C. silvarum*

	Wood	Parmelia sulcata v.	Parmelia physodes d.	Parmelia physodes v.	Leaf	Parmelia sulcata d.	Moss
♂	3.0 ± 0.50	[2.1 ± 0.42	⋮ 1.9 ± 0.23	1.3 ± 0.29 ⋮]	0.5 ± 0.15	0.3 ± 0.15	0.2 ± 0.06

	Wood	Leaf	Parmelia sulcata v.	Parmelia sulcata d.	Parmelia physodes v.	Parmelia physodes d.	Moss
♀	3.1 ± 0.38	[2.4 ± 0.35	⋮ 2.0 ± 0.36 ⋮]	1.0 ± 0.23	0.9 ± 0.22	0.7 ± 0.19 ⋮	0.5 ± 0.11

TABLE VIII. Food preference of *S. sabulosum*

	Parmelia physodes v.	Parmelia sulcata v.	Wood	Leaf	Parmelia physodes d.	Parmelia sulcata d.	Moss
♂	1.8 ± 0.42	1.7 ± 0.36	1.6 ± 0.39	[1.1±0.24	⋮ 0.8±0.11	0.6 ± 0.11]	0.4±0.07
♀	1.7 ± 0.31	1.6 ± 0.26	1.6 ± 0.10	1.4±0.24	[1.1 ± 0.24 ⋮	0.8 ± 0.22	0.5±0.12]

TABLE IX. Food preference of *I. scandinavius* (♂+ ♀)

Parmelia sulcata v.	Parmelia physodes v.	Parmelia sulcata d.	Moss	Parmelia physodes d.	Mold	Wood	Leaf
0.6 ± 0.13	0.6 ± 0.14	0.3 ± 0.07	0.3 ± 0.07	0.3 ± 0.08	0.1 ± 0.06	0.1 ± 0.04	0.1±0.04

There was close agreement between results with male and female *C. silvarum* and *S. sabulosum*. Because of difficulty in obtaining *I. scandinavius*, males and females of this species were not tested separately.

C. silvarum

Table VII shows that wood was among the substances most often eaten by *C. silvarum* and that moss was rarely eaten. Males exhibited preference for the two species of lichen over rotting leaves while the reverse was true with the females. The preference for leaves and lichens was further analysed in separate tests, with animals of both sexes, which produced the following results.

Leaves. — Three species of rotting leaves in order of preference and the mean values of all the observations were: *B. verrucosa*, 5.6 ± 0.32, *P. tremula*, 4.6 ±0.29, *C. monogyna*, 0.4 ± 0.10.

Lichens. — A series of tests with the two species of lichens supported the result, shown in Table VII, that no constant preference was exhibited for either one of the lichens over the other.

Mold. — Tests including the mold, *Aspergillus* sp., as one of the foods yielded the following results.

Wood	Leaf	Parmelia sulcata v.	Parmelia sulcata d.	Parmelia physodes d.	Parmelia physodes v.	Mold
2.2 ± 0.34	2.0 ± 0.24	1.0 ± 0.13	0.6 ± 0.16	0.2 ± 0.09	0.1 ± 0.07	0.1 ± 0.07

Very little mold was eaten although it was consumed in abundance when no other food was available and also was found regularly in feces from animals in the field. In these tests, *P. sulcata* was preferred over *P. physodes*.

S. sabulosum

Lichens were eaten equally as much as wood by this species which also exhibited a general preference for the ventral surfaces of the lichens over the upper surfaces. Moss was eaten seldom as was the case with *C. silvarum*. The results of subsequent tests were as follows.

Leaves. — Rotting *B. verrucosa* leaves (4.6 ± 0.55) took first place over rotting *C. monogyna* (3.2 ± 0.32) and *P. tremula* (2.4 ± 0.27) leaves. Contrary to previous observations, *C. monogyna* leaves were eaten during these tests.

Lichens. — No difference in preference between the two lichens was found and although a preference for the ventral surface of *P. sulcata* over the dorsal surface was shown, no difference was found with *P. physodes*.

Mold. — The mean values for tests using *Aspergillus* sp. as one of the foods were:

Parmelia sulcata v.	Parmelia physodes v.	Parmelia sulcata d.	Lea	Mold	Wood	Parmelia physodes d.
1.8 ± 0.22	1.4 ± 0.26	1.3 ± 0.25	0.9 ± 0.18	0.8 ± 0.19	0.5 ± 0.12	0.4 ± 0.11

Here also, statistical comparison of the means has not much value because of the huge standard errors. But the results tend to indicate that *S. sabulosum* showed no particular preference for this species of mold as food. The general trend of the results is similar to that of Table VIII.

I. scandinavius

Feeding activity was less with this species than with the other two species as shown by the low mean values in Table IX. Individuals of this species are relatively very excitable and it is possible that handling of the animals at the start of an experiment and opening and closing of the lid of the box during observations had a more disturbing effect than in the case of the other two species.

Green plant material in the form of lichens and moss was eaten more often than mold, wood, and leaves. This observation is somewhat contrary to results obtained from examination of feces from animals in the field where leaves apparently form an important part of the diet.

Leaves. — Experiments with the three species of leaves resulted in a pronounced preference for *B. verrucosa* (2.4 ± 0.37) over *P. tremula* (0.6 ± 0.25) and *C. monogyna* (0.1 ± 0.07).

Influence of Light and Darkness and Variety of Diet

Examination of feces from animals in the field had revealed the remains of more green plant material than wood in the excrement of *C. silvarum* and *S. sabulosum* collected at night. In an attempt to reproduce this result in the laboratory, individuals of these two species were offered a choice of wood and lichen and the composition of feces produced by them during the day and at night was determined. Although the percentage of lichen found in feces produced at night was slightly greater than during the day, the difference was not statistically significant. Proof of the existence of a diurnal feeding rhythm was thus not obtained. It is assumed that the previously observed difference in composition of feces produced during the day and at night resulted from the technique of collection. Animals climbing on tree trunks at night received particular attention although they probably represented only a small proportion of the population in the area.

The influence of a constant diet of one particular substance on subsequent food preference was also examined with *S. sabulosum* and *C. silvarum*. In a series of tests, individuals were fed only one food before and between the experiments and were offered a choice between that same substance and another during the tests. Preference was determined, as in previous experiments, by observing the positions of the animals at regular intervals. Results obtained with *S. sabulosum* are presented in Table X. Figures represent the mean number of animals observed on each food.

TABLE X. Influence of a constant diet on food preference of *S. sabulosum*

(a) Animals fed *P. physodes* before and between tests

Wood	*P. physodes*
1.3 ± 0.16	0.7 ± 0.16

(b) Animals fed wood before and between tests

P. physodes	Wood
1.8 ± 0.31	0.8 ± 0.21

(c) Animals fed wood before and between tests

P. sulcata	Wood
2.2 ± 0.27	0.7 ± 0.19

The results are fairly convincing. A constant diet of one particular substance produced a subsequent tendency to reject this substance when a choice was possible. A similar relationship was not obtained with *C. silvarum*. In tests with this species in which wood and leaves were used, wood always received slight preference over leaves regardless of the diet before and between the tests. It may be assumed that *S. sabulosum* eats a more varied diet than *C. silvarum* for which species wood is an important food. The food preference of *C. silvarum* is also somewhat stronger than that of *S. sabulosum*. This is suggested also by the results given in Tables VII and VIII.

SUMMARY AND CONCLUSIONS

Rotting leaves and rotting wood appear to be the most generally accepted foods of *C. silvarum, S. sabulosum,* and *I. scandinavius*. Living plant material in the form of lichens, mosses, and algae, is also eaten.

Living and intact, dead wood is not accepted as food. Wood has to be soft and rotting before it is eaten. Fresh, green leaves of deciduous trees are also rejected.

The factors responsible for differences in palatability of different substances were not determined but must be rather general because of the wide variety of substances eaten.

The digestion process is very incomplete. Chewing is superficial so that relatively large fragments pass the mandibles. Substances are apparently little altered by passage through the gut. No evidence was found of the digestion of either cellulose or lignin.

Food preference was limited only to groups of substances. No dominant preference for one particular food was exhibited by any of the three species. Consequently, it is unlikely that food is an important factor in their distributions.

In laboratory experiments, *C. silvarum* ate the two lichens, *P. sulcata* and *P. physodes*, somewhat less than rotting wood and rotting leaves. *S. sabulosum* and *I. scandinavius* ate these substances slightly more than rotting wood and rotting leaves. However, examination of feces from animals in the field indicated that leaves form an important percentage of the diet of *I. scandinavius*.

All three species of millipedes chose rotting leaves of *B. verrucosa* over rotting leaves of *P. tremula* and *C. monogyna*.

With *S. sabulosum*, a constant diet of a certain substance caused a subsequent rejection of this food in favour of others. This result was not obtained with *C. silvarum*. The diet of *S. sabulosum* is probably more varied, in accordance with its wide distribution, than that of *C. silvarum* for which wood is an important food and which, correspondingly, is rarely found in other than wooded terrain.

COMBINATIONS OF FACTORS

It has already been emphasized that an animal under natural conditions is never exposed to a single, isolated, environmental factor but to a continually changing complex of biotic and abiotic elements. The reaction of an individual to combined factors may not necessarily be the same as the sum of its responses to each factor acting independently. Whether or not this is true must ultimately be determined by examining the effects of combinations of factors.

Of the three factors considered, humidity and temperature are apparently of greatest influence in the distributions of these millipedes. An opportunity to observe the joint action of these two elements is given by the data presented in Figs. 4, 5, and 6. Results of the foregoing experiments have shown that periods of greatest activity of the millipedes are associated often with strongest resistance to desiccation and lowest intensity of attraction to high humidity. Retention of body water, by one means or another, is continually necessary for these animals. Figs. 4—6 show, however, that *I. scandinavius* and *C. silvarum* are relatively inactive in summer when the greatest amount of rainfall is recorded. In the following paragraphs, the relationship between temperature, humidity (precipitation), and activity are examined at the level of the existing data.

METHODS OF ANALYSIS

Hygrothermographs

Data from Figs. 4—6 have been inspected in two ways. The first method is an analysis of what constitutes a "peak" in activity and a "low" in activity in relation to temperature and precipitation by constructing hygrothermographs for the data. These figures give an impression of the conditions of temperature and precipitation concurrent with a certain biological phenomenon, in this case, maximum and minimum activity.

To obtain an objective criterion of peak and low activity which also conformed to the obvious character of the data, the mean number of animals trapped per month was calculated for each year; a "peak" in any year was then taken as anything more than the mean catch plus one standard error and a "low" as anything less than the mean catch minus one standard error. The use of one standard error as difference factor applies only to this particular series of graphs. Conditions of mean temperature and total precipitation recorded for months thus designated as "peaks" or "lows" were plotted with precipitation as abscissa and temperature as ordinate. A number of points constituting any single "peak" or "low" were joined in temporal succession. The resulting enclosed area, line or single point, indicates the temperature and precipitation which existed, as well as the magnitudes of changes which occurred in both these factors, during the so designated period.

Partial Regression

The second method of analysis applied to the data was that of partial regression, the purpose of which is to obtain a function of Y, the dependent variate, in terms of a number of independent variates, x_1, x_2 ……, which are believed to affect Y and thus to determine the average effect of each factor on Y. A formula of the form, $Y = b_1x_1 + b_2x_2 + \ldots\ldots b_px_p$, is required where b_1, b_2, …… b_p are the partial regression coefficients of the corresponding independent variates. The algebraic procedure for deriving and using this formula involves use of the covariance matrix and has been explained by Fisher (1954). For the data at hand, the influence of monthly mean temperature and total precipitation per month on the number of animals trapped (activity) each month have been determined.

When calculating correlations such as these, it is important to keep in mind the obvious biological facts which, in this case, are first, that activity periods are cyclic, and second, that changes in activity at any time may be partly positively and partly negatively correlated with contemporary fluctuations in temperature and precipitation. Different sections of the yearly cycle should thus be considered separately. Primary among the advantages of the present statistical method are that it enables the user to do this as well as to calculate regressions for different species on the same conditions of temperature and precipitation without having to recalculate completely the formula for these factors each time.

RESULTS AND DISCUSSION

Figs. 45, 46, and 47, are hygrothermographs constructed for each of the three species. Since little intraspecific difference in activity was recorded, data for males and females were combined in the graphs. A favourable amount of regularity is obvious between the distributions of comparative "peaks" and "lows" during different years.

C. silvarum. — Spring peaks in activity of this species were always associated with moderate mean temperature and low to moderate rainfall. Inactivity during summer occurred in periods of relatively high mean temperature and usually moderate, but in 1955 low, rainfall. Moderate to high mean temperatures were common during autumn activity peaks. But these peaks, except in 1955, occurred in conjunction with heavy rainfall. Winter inactivity was always combined with low to moderate mean temperature and moderate precipitation.

I. scandinavius. — Moderate mean temperatures and low to moderate rainfall were typical during spring activity peaks in this species too. Summer low activity usually occurred together with relatively high mean temperature and moderate to high rainfall. An autumn peak activity was exhibited by this species only in 1953 together with moderate temperature and low rainfall. The position of this point on the graph indicates close similarity between conditions of temperature and rainfall during autumn and spring peaks in that year. Actually, on the basis of temperature and rainfall, no separate peak should be recognized. Again with this species, winter low activity was associated with low to moderate mean monthly temperature and moderate precipitation.

S. sabulosum. — This species exhibited only one "peak" and one period of relative inactivity per year. "Peaks" occurred in conjunction with moderate to high

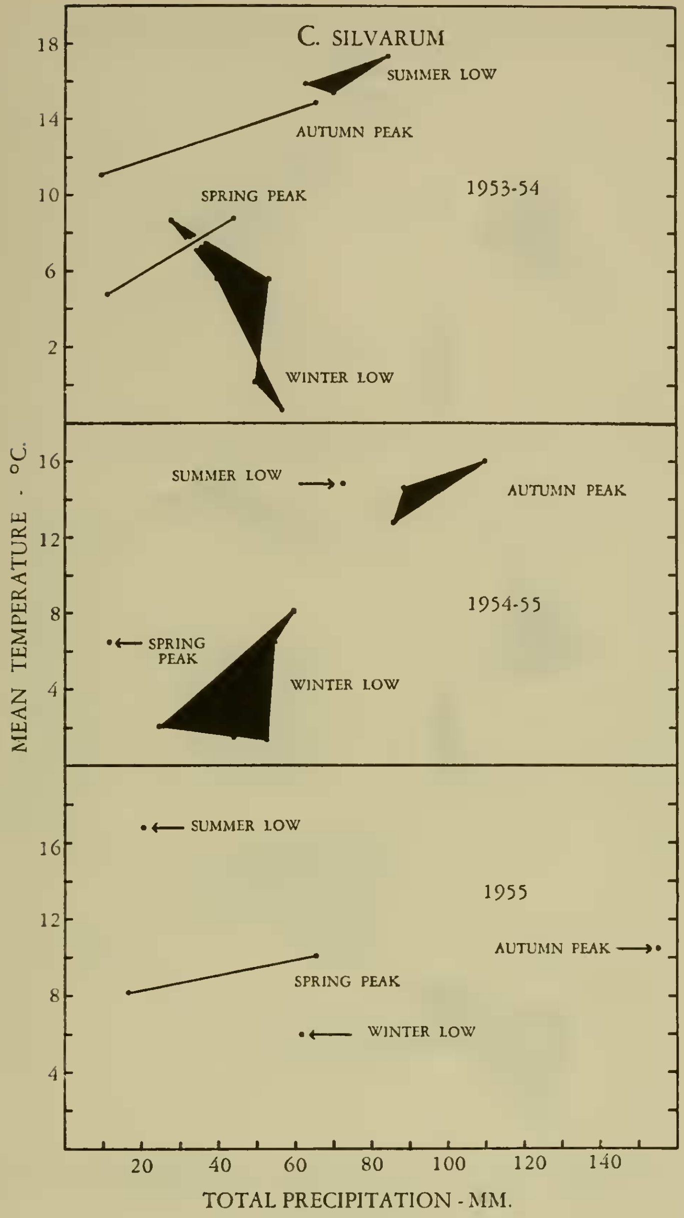

Fig. 45. Hygrothermographs for periods of maximum and minimum activity of *C. silvarum* during three consecutive years (c.f. Fig. 5)

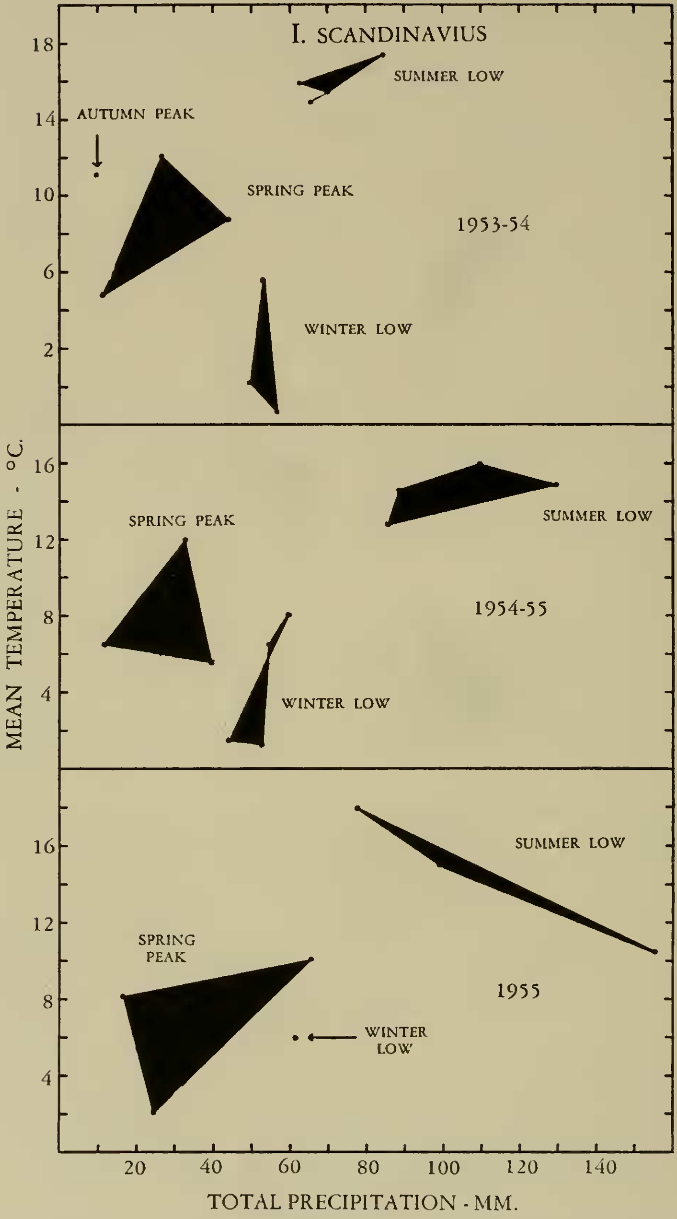

Fig. 46. Hygrothermographs for periods of maximum and minimum activity of *I. scandinavius* during three consecutive years (c.f. Fig. 4)

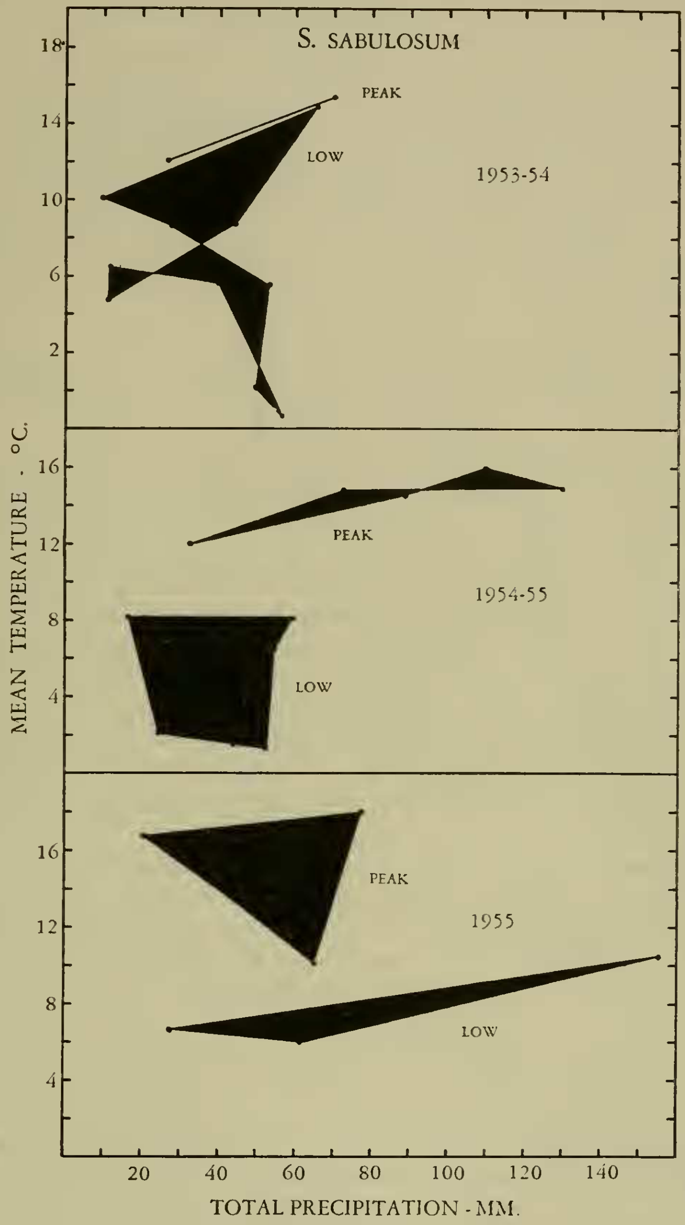

Fig. 47. Hygrothermographs for periods of maximum and minimum activity of *S. sabulosum* during three consecutive years (c.f. Fig. 6)

mean temperature and a wide range of rainfall. The inactive period was characterized by low to moderate mean temperature and usually low to moderate precipitation but in 1955 included also heavy rainfall.

When viewed together, these results substantiate a number of relationships between temperature, precipitation, and activity optima in the three species. Maximum activity of C. *silvarum* may occur, in the field, during periods of either moderate or high mean temperature but when the mean temperature per month is high, rainfall is also moderate to relatively high and never low. This fact suggests that a large amount of rainfall may compensate for high temperature by producing conditions of dampness suitable for optimum activity. Without this high rainfall, conditions of relatively high temperature become inhibitory to increased activity.

The compensating effect of high rainfall does not exist with *I. scandinavius*. This species shows maximum activity only under conditions of moderate temperature and low to moderate rainfall. Relatively high temperature may be inhibitory either in itself or because associated conditions of rainfall are not sufficiently heavy to produce moisture conditions suitable for maximum activity of *I. scandinavius* at these relatively high temperatures. It has already been shown that, of the three species, *I. scandinavius* is most susceptible to water loss.

Maximum and minimum activity of S. *sabulosum* are characteristic of a much wider range of temperature and particularly of precipitation. Peak activity of this species occurs in conjunction with moderate to high mean monthly temperature regardless of the amount of rainfall. This agrees well with the eurytopic nature of the species and the fact that it has a relatively strong resistance to high temperature and to water loss.

In all three species winter inactivity is combined with low to moderate mean monthly temperature and, generally, low to moderate precipitation. Because of the length of this period in S. *sabulosum*, it may also include intervals of heavy precipitation.

For purposes of this analysis, any mean monthly temperature below about 4° is considered as low and any mean temperature above 12° as high. Moderate temperatures are between these two. Less than 30 mm total precipitation per month is designated as low and more than approximately 80 mm per month as high or heavy. Total monthly precipitation between these two figures is termed moderate.

These results have supplied some insight into the nature of activity maxima and minima. To carry the analysis a step further, information is required about the nature of increases and decreases in activity in relation to temperature and precipitation and the relative influence of these two factors on these activity changes. The method of partial regression was applied to the data to try to extract this information. Results of this analysis are given in Table XI in which "b_1" and "b_2" are the partial regression coefficients of mean monthly temperature and total monthly precipitation, respectively, "R" is the total regression of these two independent variates, "$(b_1—b_2) \pm$ S.E." is the difference between "b_1" and "b_2" with the standard error of this difference, and "p" is the probability that the magnitudes of "b_1" and "b_2" are significantly different.

Figures in this table, clearly, do not warrant valid conclusions concerning the

TABLE XI. Statistical analysis of the influence of changes in mean monthly temperature and total monthly precipitation on contemporary changes in the number of animals trapped (activity)

Activity period	No. of obs'ns	b_1	b_2	R	$(b_1 - b_2) \pm$ S.E.	p
		C. silvarum				
Spring increase	6	0.14	0.002	0.14	0.14 ± 0.711	0.8+
Summer decrease	9	−0.24	0.02	0.19	0.26 ± 0.528	0.6−
Autumn increase	11	−0.28	0.05	0.41	0.33 ± 0.306	0.3+
Autumn decrease	6	0.01	0.05	0.38	0.04 ± 0.513	0.9
		I. scandinavius				
Spring increase	8	0.41	0.04	0.23	0.37 ± 0.701	0.6
Summer decrease	13	−0.11	−0.05	0.47	0.06 ± 0.255	0.8
Autumn increase	6	−0.10	0.02	0.24	0.12 ± 0.284	0.7
Autumn decrease	6	0.16	−0.02	0.39	0.18 ± 0.256	0.5
		S. sabulosum				
Spring increase	8	0.28	0.01	0.16	0.27 ± 0.900	0.7 +
Autumn decrease	18	0.36	0.00	0.41	0.36 ± 0.248	0.2−

combined effect of temperature and precipitation on the number of animals trapped or the relative influence of each factor on changes in the catch. Random variations in the data were too great.

Although the regression coefficients are not significant, a number of regularities in the figures are worth mention. Almost without exception, the regression coefficient of temperature (b_1) was larger than that of precipitation (b_2) for the same period. Secondly, spring increases and autumn decreases in activity of *I. scandinavius* and *C. silvarum* usually produced positive coefficients. Summer decreases and autumn in increases gave negative temperature coefficients. While concluding nothing about the influence of either temperature or precipitation or both factors on changes in activity, the signs of the coefficients suggest that increases of activity of these two species during spring occurred while temperature and rainfall were also increasing, on the average. When temperature and rainfall continued to increase during summer, activity decreased. While activity was increasing in autumn, temperature was decreasing and as temperature continued to decrease during fall, activity also decreased. The coefficients were positive with *S. sabulosum*. Activity of this species increased while temperature was increasing and decreased while temperature was decreasing. These relations are suggested also by Fig. 4—6. A third point of perhaps considerable importance is that the "p" values of the differences between the coefficients decrease fairly regularly with increasing numbers of observations. This fact implies that, if the observations were numerous enough, the suggested relationships would prove statistically reliable. Data such as these should be studied at weekly, instead of monthly, intervals.

With the existing data, statistical analysis has given no valid indication of the influence of either temperature or precipitation on changes in activity of these millipedes during the year. However, it is evident from the hygrothermographs that the occurrence of activity maxima and minima are related to associations of these factors which are distinct for each species.

REACTIVITY AND DISTRIBUTION

The distribution of an animal species is ordinarily a function of the plasticity of the species or the degree to which its members are influenced by environmental factors. In the present study, the distributions of *S. sabulosum* and *C. silvarum* generally agreed with their reactions to humidity and temperature. *S. sabulosum* is a species with a wide distribution; it possesses strong resistance to water loss at low humidities and is relatively unrestricted in its movements by the temperature of its environment. *C. silvarum,* on the other hand, occurs in a more confined area and is more strongly affected by temperature and humidity than *S. sabulosum* is. The case of *I. scandinavius*, however, is anomalous to this relationship. This species, while being highly susceptible to environmental extremes, and showing strong humidity and temperature preferences, nevertheless has a much more extensive distribution than *C. silvarum* and is almost as widespread as *S. sabulosum.* But *I. scandinavius* is a relatively active animal, this characteristic activity or excitability being readily observable when handling individuals of the species and in their reactions towards humidity and temperature and it seemed feasible that this quality might make possible its wide range even though it is unable to withstand environmental extremes.

To illustrate this point, the relative reactivity of the millipedes to light was determined. All three species are more or less negatively phototropic, the presence of light causing various avoiding reactions in the animals. But light, itself, has no obvious harmful effects on the individuals; it is merely a „token stimulus" (MAST, 1911), changes in its intensity under natural conditions usually indicating corresponding changes in humidity and temperature and generally in protective cover. Thus the behaviour of the millipedes towards light is purely an avoiding reaction depending on the excitability or reactivity of the animals uncomplicated by any specific faculty for withstanding injurious conditions. Light was therefore chosen as a medium for expressing, quantitatively, the characteristic reactivity potential of each species.

METHOD.

The kinetic response of the millipedes to a constant light intensity was determined by observing the distance in centimeters travelled by individuals during five minutes' exposure to light from a 100W overhead lamp at a distance of 15 cm. The animals were placed in a length of transparent plastic tube which was marked off in centimeters. The ends of the tube were then joined forming a circular runway for the millipede inside. The 100W lamp was mounted directly above the mid-point of the circle formed by the runway. Experiments were carried out in a photographic darkroom and each animal was allowed to remain five minutes in the tube in total darkness before observations were made. This length of time was sufficient for the individual to become stationary after handling. The lamp was then switched on and the distance travelled by the animal in five minutes was noted. A thermometer was placed in the circle formed by the plastic tube and